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Interspecies Extrapolations of Halocarbon Respiratory and Tissue Kinetics: Applications to Predicting Toxicity in Different Species

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A series of experiments have been conducted to provide a pharmacokinetic data base for interspecies comparisons and for formulation and validation of physiologically-based pharmacokinetic models. The basic experimental design has involved giving equal doses of halocarbons to the rat and the dog, two species of widely different size. Perchloroethylene (PCE), tetrachloroethane (TET), trichloroethylene (TCE), and trichloroethane (TRI) have been employed as test chemicals, in order to evaluate the relative importance of the physicochemical property of volatility on the kinetics and toxicity of halocarbons. The respiratory elimination of TCE and systemic uptake of TCE and PCE has been measured in rats. In order to determine the dose received in target organs and other tissues, serial samples of brain, liver, kidney, lung, heart, skeletal muscle, and adipose tissue have been taken and analyzed for halocarbon content after administration of PCE, TET, and TRI in rats, and PCE and TET in dogs. A neurobehavioral operant testing system has been set up and a protocol established for monitoring the central nervous system effects of halocarbons. Neurobehavioral studies have
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been conducted following oral and inhalation exposure to PCE, and from inhalation exposure to TRI in rats. The direct measurements of halocarbon concentrations in exhaled breath and tissues have provided an extensive data base that will be used to formulate and validate the physiologically-based pharmacokinetic (PBPK) models for exposure to halocarbons. The blood and exhaled breath kinetics of PCE and TCE were accurately simulated by PBPK models. The physiological parameters for PBPK model formulation in the dog have also been procured in an extensive literature search. The partition coefficients for developing PBPK models for rats and dogs were determined from detailed tissue determinations in these species.

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TECHNICAL SUMMARY

A series of experiments have been conducted to provide a pharmacokinetic data base for interspecies comparisons and for formulation and validation of physiologically-based pharmacokinetic models. The basic experimental design has involved giving equal doses of halocarbons to the rat and the dog, two species of widely different size. Perchloroethylene (PCE), tetrachloroethane (TET), trichloroethylene (TCE), and trichloroethane (TRI) have been employed as test chemicals, in order to evaluate the relative importance of the physicochemical property of volatility on the kinetics and toxicity of halocarbons. The respiratory elimination of TCE and systemic uptake of TCE and PCE has been measured in rats. In order to determine the dose received in target organs and other tissues, serial samples of brain, liver, kidney, lung, heart, skeletal muscle, and adipose tissue have been taken and analyzed for halocarbon content after administration of PCE, TET, and TRI in rats, and PCE and TET in dogs. A neurobehavioral operant testing system has been set up and a protocol established for monitoring the central nervous system effects of halocarbons. Neurobehavioral studies have been conducted following oral and inhalation exposure to PCE, and from inhalation exposure to TRI in rats. The direct measurements of halocarbon concentrations in exhaled breath and tissues have provided an extensive data base that will be used to formulate and validate the physiologically-based pharmacokinetic (PBPK) models for exposure to halocarbons. The blood and exhaled breath kinetics of PCE and TCE were accurately simulated by PBPK models. The physiological parameters for PBPK model formulation in the dog have also been procured in an extensive literature search. The partition coefficients for developing PBPK models for rats and dogs were determined from detailed tissue determinations in these species.

I. OVERALL OBJECTIVE AND SPECIFIC AIMS

The overall objective of this project is to investigate the scientific basis for the interspecies extrapolation of pharmacokinetic and neurobehavioral toxicity data. Direct measurements of the respiratory elimination and tissue concentrations of halocarbons over time in two species will be used to formulate and validate physiologically-based pharmacokinetic models for halocarbon exposure. These models will be used for: (a) prediction of the time-course of the respiratory elimination and target organ levels of halocarbons; (b) interspecies extrapolations (i.e., scale-up from smaller to larger laboratory animals and ultimately to man). A combined physiological pharmacokinetic-toxicodynamic model for halocarbon exposure will subsequently be developed and evaluated for its ability to predict neurobehavioral effects under specified exposure conditions.

SPECIFIC AIMS in the proposed studies are to:

1) Determine the respiratory elimination of physicochemically disparate volatile organic compounds (VOCs) in two animal species. Rats and dogs will be administered selected halocarbons by inhalation and oral exposure. Concentrations of expired parent compounds will be monitored in the exhaled breath for appropriate periods during and following exposure. Together with monitoring of the respiratory volumes of the test animals, this data will enable calculation of the cumulative uptake and elimination of the halocarbons. Data from both species will be compared to existing data sets for respiratory elimination in humans.

2) Delineate the tissue disposition of inhaled halocarbons in two animal species. Rats and dogs will be exposed to halocarbons by inhalation. Concentrations of the parent compound in brain, liver, heart, lung, kidney, skeletal muscle, and adipose tissue will be measured over time, in order to provide an assessment of the actual target organ dose for correlation with neurobehavioral toxicity and for development and validation of physiologically-based pharmacokinetic and toxicodynamic models.

3) Validate physiologically-based pharmacokinetic (PBPK) models for predicting the tissue pharmacokinetics of halocarbons in two animal species. PBPK models that have been initially developed in rats in our previous studies, with blood and expired air data for inhalation exposures and with tissue data for intraarterial and oral exposures, will be further validated for accuracy in interspecies extrapolations. Direct measurements of exhaled breath and tissue concentrations and associated parameters in rats and dogs in the initial two phases of the presently proposed project will be used to further test the accuracy of our PBPK models. Models will be developed each for the rat and the dog. The observed animal data and pharmacokinetic parameters will be used to formulate allometric relationships which can then be used to predict human disposition of halocarbons.

4) Correlate the neurobehavioral toxicity of inhaled VOCs in two species with the target organ concentration. Rats and dogs will be exposed to selected

halocarbons at defined inhaled concentrations and lengths of exposure. Neurobehavioral tests for operant performance will be performed periodically during and after exposures. The magnitude of central nervous system (CNS) effects of each solvent will be correlated with the target organ (i.e., brain) halocarbon concentration, as determined in (2), at each time-point. Thereby, it will be possible to determine whether equivalent target organ doses in the rats and dogs elicit CNS effects of comparable magnitude in each species.

5) Develop and validate toxicodynamic models for inhaled halocarbons. Brain halocarbon concentrations will be correlated with the magnitude of neurobehavioral toxicity in an appropriate equation. These relationships will be used in conjunction with the PBPK model developed in (3). The CNS effects observed in (4) will be compared to predicted effects to assess the validity of the model in the two species tested. Validated models may allow the prediction of CNS effects over time of exposure using: a) extrapolations from pharmacokinetic data; b) simulations in the absence of experimental data.

II. FUNDAMENTAL HYPOTHESIS TESTING

A very important question faced by scientists and administrators conducting risk assessments is the relevance of toxic effects seen in animals to anticipated adverse effects in humans. Pharmacokinetic studies are playing an increasingly important role in species to species extrapolations in toxicology. Blood concentration over time, as a measure of bioavailability, is routinely accepted as an index of the level of chemical in the entire system, and therefore a representative parameter of toxic effects. This assumption can be misleading, in that blood concentrations may not be reflective of the concentration of chemical or active metabolite at the local site of effect in a target organ or tissue. It is now recognized that chemical toxicity is a dynamic process, in which the degree and duration of toxic effect in each tissue is dependent upon systemic absorption, tissue distribution, interactions with cellular components, and clearance from the tissue and body by metabolism and excretion. Estimation of the risk of toxic injury from pharmacokinetic data is based on the assumption that the intensity of the response from a given dose is dependent upon the magnitude of the dose received by a target tissue. A related assumption can be stated in the form of a HYPOTHESIS:

THE DOSE RECEIVED IN A PARTICULAR TARGET TISSUE IN ONE SPECIES WILL HAVE THE SAME DEGREE OF EFFECT AS AN EQUIVALENT TARGET TISSUE DOSE IN A SECOND SPECIES.

There are surprisingly few scientific data that are applicable to this basic assumption, although it is a very important premise in interspecies extrapolations in toxicology. If valid, it is a logical basis on which to evaluate the suitability of different species as predictors of toxicity in humans (i.e., the species in which target organ deposition is most similar to man would likely be an appropriate animal model for toxicity testing).

A series of parallel studies in different animal species is therefore being employed to test the foregoing hypothesis. The rat and dog have been subjected

to equivalent exposure to halocarbons. Similarities and differences in respiratory elimination, tissue disposition, and toxicity between the species are being determined. Neurobehavioral alterations will be used as a toxic end-point in the currently proposed work, since: a) OSSA and EPA commonly use neurobehavioral effects as the basis for deriving standards for exposure to VOCs; b) central nervous system (CNS) depression is caused by and can be directly correlated with the concentration of parent compound in the CNS (Bruckner and Peterson, 1981).

III. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL DEVELOPMENT FOR RESPIRATORY ELIMINATION AND SYSTEMIC UPTAKE OF TCE IN RATS

A paper has been published during the first year of this project on the pharmacokinetics and PBPK model validation for inhaled TCE. The reprint is included as Section A of the Appendix, and the reference is as follows:

> Dallas, C.E., Gallo, J.M., Ramanathan, R., Muralidhara, S., and Bruckner, J.V. "Physiological pharmacokinetic modeling of inhaled trichloroethylene in rats." Toxicology and Applied Pharmacology 110: 303-314 (1992).

The pharmacokinetics of trichloroethylene (TCE) was characterized during and following inhalation exposures of male Sprague-Dawley rats. The blood and exhaled breath TCE time-course data were used to formulate and assess the accuracy of predictions of a physiologically based pharmacokinetic (PB-PK) model for TCE inhalation. Fifty or 500 ppm of TCE was inhaled by unanesthetized rats of 325-375 g for 2 hr through a miniaturized one-way breathing valve. Repetitive samples of the inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently during and for 3 hr following the exposures and analyzed for TCE by headspace gas chromatography. Respiratory rates and volumes were continuously monitored and used in conjunction with the pharmacokinetic data to delineate uptake and elimination profiles.

TCE exhaled breath levels were found to have increased rapidly after the initiation of exposure to near steady-state within approximately 20-30 min and were then directly proportional to the exposure concentration. Uptake of TCE in the blood was also rapid, but blood levels continued to increase progressively over the course of the inhalation exposure at both dose levels. Arterial TCE concentrations were not proportional to the inhalation concentration, with levels for the 500 ppm group from 25-30 times greater than in 50 ppm-exposed rats during the second hour of the exposure. Percent uptake rapidly thereafter until equilibrium was established by 1 hr, after which % uptake of the inhaled dose was approximately 69-72% for both exposure groups. Total cumulative uptake of 50 to 500 ppm TRI over the 2-hr inhalation exposures was determined to be 8.4 and 73.3 mg/kg bw, respectively. The direct measurements of TCE in the blood and exhaled breath were utilized in the validation of a physiological pharmacokinetic model of the prediction of the pharmacokinetics of inhaled TCE. Results from this study indicate that metabolism of TCE is saturable between 50 and 500 ppm exposure in rats, resulting in disproportionately higher blood levels above the saturation point. At doses below this metabolism saturation point in rats, blood and exhaled breath levels of TCE in rats were very similar to values previously published for TCE inhalation exposures in humans. The PB-PK model was

characterized as blood flow-limited with TCE eliminated unchanged in the exhaled breath and by saturable liver metabolism. The uptake and elimination profiles were accurately simulated by the PB-PK model for both the 50 and 500 ppm TCE exposure levels. Such a model may be quite useful in risk assessments in predicting internal (i.e., systemically absorbed) doses of TCE and other volatile organics under a variety of exposure scenarios.

IV. ANALYTICAL DETERMINATIONS OF HALOETHANES AND HALOETHENES IN TISSUES

A manuscript has been submitted to a scientific journal on the method that was developed for tissue determinations of aliphatic halocarbons. This submitted manuscript is included as section B of the appendix, and the reference is as follows:

> Chen, X.M., Dallas, C.E., Muralidhara, S., Srivatsan, V., and Bruckner, J.V. "Determination of volatile halogenated hydrocarbons in body tissues." Submitted to Journal of Chromatography (1992).

Characterization of the systemic uptake, distribution and elimination of volatile organic compounds (VOCs) requires reliable analytical techniques for measuring the concentration of the chemicals in different tissues of the body. An extraction procedure was developed which minimized loss of the readily volatilizable compounds, so that they could subsequently be quantified by headspace gas chromatography. The procedure was evaluated using four C2 halocarbons [i.e., perchloroethylene (PER), 1,1,1-trichloroethane (TRI), 1,1,2,2-tetrachloroethane (TET), and 1,1,2-trichloroethylene (TCE)] of varying physicochemical properties. Portions of 0.5 to 1 g of liver, kidney, brain, heart, lung, skeletal muscle, fat and blood from rats were spiked with PER to yield a theoretical concentration of 4 μg/g tissue. Two homogenization procedures were evaluated: (a) tissues were homogenized in saline, followed by isooctane extraction; and (b) tissues were homogenized in isooctane and saline (4:1, v:v). the latter approach resulted in a significantly higher percent recovery of PER from most tissues. Neither homogenization nor the presence of saline affected PER standards prepared in isooctane. It was observed that the volume of the aliquot of isooctane taken for PER analysis was important, in that aliquots >25μl could not be used. PER concentrations were determined in tissues of rats following *in vivo* (i.e., intraarterial administration) of the halocarbon using the latter (i.e., isooctane) homogenization approach. This approach was also employed to examine the efficiency of recovery of PER, TET, TRI, and TCE from seven tissues and from blood. Percent recoveries of each of the four halocarbons ranged from 73-104% for the seven spiked tissues. The recoveries did not appear to be tissue-dependent, despite differences in homogenization time required for different tissues. Recovery, however, did vary somewhat with test chemical, with the least volatile, most lipophilic compounds exhibiting the highest recovery.

Extensive analyses in two species of rats and dogs have been conducted to determine the percent recovery for both PCE and TET in the following tissues: brain, liver, kidney, lung, fat, heart, and muscle. In order to know the variation in percent recovery between species, PCE and TET chemicals were chosen to measure the percent recovery in both rats and dogs. For rats, four different

concentrations 0.1, 1, 5 and 20 mg/ml, six groups for percent recovery of dog tissue 0.1, 0.5, 1, 5, 10 and 20 mg/ml were selected. In each group, there were 6 samples for each kind of tissue. Animals were anesthetized with ether. One ml blood samples were withdrawn by closed cardiac puncture. Approximately 1 gram of rat tissue were removed and placed on ice. In case of dog tissue, a dog was sacrificed and all recoverable tissue types were harvested and divided into small pieces which were approximately equal to 1 gram. PCE and TET at concentrations of 0.1, 1, 5, and 20 mg/ml for 4 groups of rats. 0.1, 0.5, 1, 5, 10 and 20 mg/ml for 6 groups of dogs were carefully injected into each tissue by using a Hamilton microsyringe. After injecting, the tissues were immediately transferred into 2 ml of chilled saline and 8 ml of chilled isooctane. The samples were maintained on ice even during homogenization. A polytron tissumizer was used to homogenize the tissue samples. Samples were extracted with 8 ml of isooctane and vortexed for 30 seconds. Samples were then centrifuged at 1800 G for 5 min at 4°C in a Sorvall RC 2-B centrifuge. Twenty ul of the organic phase was withdrawn with an Eppendorf pipet and transferred to 20 ml headspace vials (Perkin-Elmer Model 8500).

Standards were made and assayed by diluting a calculated amount of pure test chemical in the appropriate solvent. The column used was 8' x 1/8" stainless-steel column packed with 3 to OV-17 on chromasorb W (100-120 mesh) operating temperatures were: injection port 200°C; electron capture detector 360°C; column 110°C for PCE, 140°C for TET; and headspace control unit 90°C.

Efficiency of recovery from rat and dog tissues for PCE and TET ranged from 87.78% to 97.8% (Table B-1.2). Low standard deviation ranging from 1.44 to 8.85 were obtained in 4 or 6 groups of rat and dog tissues. There was no big difference between the lowest concentration 0.1 mg/ml and the highest 20 mg/ml. In the two compounds and the two species of animals. Comparison of different kind of tissues of rat and dog indicated that the percent recovery was very consistent for PCE, for TET kidney, fat and muscle of rat tissue recovery were slight lower than dog's. Between PCE and TET, recoveries of PCE were a little higher than TET. In summary, efficiency of recovery at various concentrations, for both chemicals and the animals were very consistent and reproducible.

V. FURTHER DEVELOPMENT OF EXTRACTION EFFICIENCY IN TISSUE HALOCARBON MEASUREMENTS

A review of the literature revealed a paucity of methods for analysis of VOCs in organs. Currently available method(s) for measuring VOCs in tissues typically involve homogenization which invariably leads to loss of these volatile compounds due to mechanical agitation and heat buildup. A new method was developed using potassium hydroxide (KOH) solution as a dissolution medium. This approach appears to improve the overall efficiency and percent recovery of VOCs over methods currently in use.

These results were presented at the most recent meeting of the Society of Toxicology. The reference for the abstract for this presentation is:

Muralidhara, S., Srivatsan, V., Sanzgiri, U., Dallas, C.E., and Bruckner, J.V. "Improvement of extraction efficiency in analysis of volatile

organic compounds (VOCs) in tissues." 31st Annual Meeting of the Society of Toxicology, Seattle, WA; Toxicologist 12: 424, 1992.

Methods currently in use in quantitate VOCs in tissues have the inherent problem of volatilization of the compounds during processing. A new technique employing KOH dissolution was developed to increase the efficiency of VOC recovery. This approach was compared to a previous method involving tissue homogenization in saline and isooctane. Percent recoveries of trichloroethylene (TCE), 1,1,1-trichloroethane (TRI), carbon tetrachloride (CCl_4) and perchloroethylene from spiked tissues were determined for the two methods. A specific amount of each VOC was injected into fresh, chilled samples of liver, kidney, fat, skeletal muscle, fat, heart, GI tract, spleen and brain taken from male S-D rats. Other rats were gavaged with an aqueous emulsion of 6 mg TRI/kg bw. The rats were sacrificed 10 min post dosing and the same organs removed. Tissue samples were treated in two ways: (1) diced in vials containing 2 ml 10N KOH and 8 ml isooctane, capped and allowed to stand for 1 hr, and vortexed; (2) homogenized in vials containing 2 ml saline and 8 ml isooctane. All of the samples were then centrifuged at 4°C at 1,900 x g for 10 min, and aliquots of the isooctane analyzed for VOC content by GC headspace analysis. Use of the KOH dissolution technique resulted in a higher % recovery from spiked samples than did the homogenization procedure for all 4 VOCs. Recovery of TCE by the KOH dissolution method, for example, ranged from 91% for fat to 105% for the GI tract. Recovery of CCl_4 using KOH was in the order of 95-100% for all tissues. TRI concentrations in tissues of rats gavaged with the VOC were consistently higher when the KOH procedure was employed. These findings indicate that KOH dissolution precludes the necessity of homogenizing tissues, and results in more efficient extraction of VOCs.

Male Sprague-Dawley rats (Charles River Labs, NC) in the weight range of 300-400 g were used for the study. Animals were housed in cages singly or in groups of two with a 12-hour light/dark cycle and *ad libitum* food and water. After ether anesthesia, the animals were exsanguinated by decapitation or closed cardiac puncture. Approximately 1 g of the following tissues were then excised (liver, kidney, perirenal fat, heart, muscle from thorax, brain and spleen). Tissue samples were placed into 20-ml scintillation vials containing 2 ml 10 N potassium hydroxide (KOH) and 8 ml gas chromatography (GC) grade isooctane. Compounds of interest were Trichloroethane (TRI); Trichloroethylene (TCE); Perchloroethylene (PER); Tetrachloroethane (TET); Carbon tetrachloride (CCl_4) were dissolved in isooctane to yield solutions of varying concentrations ranging from 0.1 mg/ml to 20 mg/ml. The tissues were spiked by injection of known quantities of the VOCs in isooctane (max volume 8 μl), using a microliter syringe.

Parallel experiments were done to study the extraction of efficiency a homogenization method vs the KOH method. Groups of 4-6 animals were dosed with the VOCs as a 5% aqueous Emulphor® emulsion, either intragastrically by gavage or intraarterially via a surgically implanted carotid cannula. The above said tissues were removed from the animals 5-10 in post dosing and processed as described above. The tissues were allowed to solubilize at room temperature in the tightly sealed headspace vials for periods of 30 min to 1 hr. Appropriate controls were run containing only KOH and isooctane or isooctane alone. The solubilization process was facilitated by mincing the tissues with a fine pair

of scissors in the medium. Samples were then vortexed for 30 sec or longer to ensure that solubilization was complete and centrifuged at approximately 1800 x g for 10 min. A 10-ul aliquot of the supernatant was transferred into a headspace vial and capped with an aluminum cap and PTFE-lined septum. The vials were placed into a thermostatically heated chamber at 80°C and a portion of the vapor phase injected automatically into the column. The GC conditions were: 6' x 1/8" stainless steel column packed with 3 % OV 17 maintained at 80°C, injector port 150°C, electron capture detector 360°C. Standard curves were developed using isooctane solutions of VOCs over a linear range of 1 ng to 50 ng. The GC output area was used to compute the recovery of VOCs, based on the standard curve and the amount of the compound injected into each tissue. Appropriate controls were used to compensate for any VOC loss during processing.

Recoveries of TRI from the KOH-processed samples were fairly consistent across the range of concentrations used to spike tissues. The lowest recovery was seen from muscle at the lowest concentration utilized (75% @ 0.1 mg/ml). Generally there was an increase in the percent recovery as the TRI concentration increased in the spiked tissues (80-100%) (Table 1). A similar concentration-dependent pattern of recovery was seen in TCE-spiked tissues (Table 2). Percent recovery values were generally quite high (80-100%) at TCE concentrations ≥ 0.2 mg/ml (i.e. ~1.6 μg/g tissue). All the tissues exhibited consistent recovery values. Recovery from tissues of the *in vivo* dosed group did not differ significantly with the two techniques (Fig. 2). The amount of TCE in tissues of rats dosed orally with 5 mg/kg was relatively low. This can likely be attributed to the extensive metabolism of TCE vs. TRI and PER.

When the chemical was given intraarterially, it was distributed to all tissues indicating that a portion of the dose was not removed by presystemic elimination (i.e. the 6 mg/kg bolus ia dose exceeded the uptake/metabolic capacity of the liver, as well as presystemic elimination. Fat had a much greater amount of TCE than did other tissues. Substantial levels of TRI and PER were also found in fat, but liver levels were also quite high because of limited metabolism of the compounds. No statistically significant difference in TCE extraction was seen between the KOH and the homogenization method groups. Studies of tissues spiked with PER (1 mg/ml) showed good recovery, ranging from 85 to 92% (Table 3). Rats dosed orally with 10 mg PER/kg bw, showed relatively high levels of the chemical in liver, kidney and fat. Lower concentrations were measured in the other tissues. Amounts of PER extracted by the two methods were not significantly different, except in blood (Fig. 3). Carbon tetrachloride showed a very poor recovery when the chemical was given orally. Percent recovery with tissues spiked with CCl_4 yielded values comparable values to TCE, TRI and PER (i.e., high recovery). Studies with TET were less successful (data not shown). Recovery of TET from spiked tissues as well as *in vivo* studies was quite low. This can be attributed to a strong chemical interaction between TET with KOH, which effectively diminished the quantity of TET in biological systems.

The KOH extraction procedure yielded excellent recovery of TRI, TCE, PER and CCl_4 from spiked tissues (i.e., 80-100%). Percent recovery values were somewhat reduced for the lowest concentration of TCE and TRI. Recovery values were dose-independent when higher concentrations (i.e. 0.2-20 mg/ml) were utilized *in vitro*. The two methods yielded similar percent recovery values for tissues of animals dosed *in vivo*. Experiments will be conducted to determine

whether this is true for spiked tissues. The new method described herewith has distinct advantages over the currently (i.e., homogenization) method in that it is time consuming and strenuous to process a large number of samples.

VI. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL DEVELOPMENT FOR INHALED PCE

An important goal of the project has been to develop and validate physiologically-based pharmacokinetic (PBPK) models, which will allow accurate prediction of the concentration of halocarbons in blood and tissues over time following inhalation and oral exposure. The pharmacokinetic studies conducted in earlier phases of the project have thus provided a unique data base from which to formulate and test the models. Data from the direct measurements of blood and exhaled breath levels of halocarbon have been compared to simulated values calculated from mass-balance differential equations comprising the model. Thereby, the accuracy of the model has been tested by comparison to observed blood and exhaled breath concentrations.

The current investigation of the uptake and elimination of PCE in rats provided the first available data base for direct measurement of PCE in the exhaled breath and blood during inhalation exposures in rats (See Appendix A, Figures 2 and 3). A PBPK model was therefore developed to describe the disposition of PCE in the rat (Appendix A, Figure 1) using this unique opportunity for comparison of computer simulated values with these direct measurements for validation of the model

A manuscript has been prepared that is now being submitted to a scientific journal for these results. The manuscript is included in this report as Appendix C, and the reference is as follows:

> Dallas, C.E., Muralidhara, S., Ramanathan, R., Gallo, J.M., Varkonyi, P., and Bruckner, J.V. "Development of a physiologically based pharmacokinetic model for inhaled perchloroethylene in rats," now being submitted to Toxicology and Applied Pharmacology.

The pharmacokinetics of perchloroethylene (PCE) was studied in male Sprague-Dawley rats to characterize and quantify systemic uptake and respiratory elimination by direct measurements of the inhaled compound. Fifty or 500 ppm per was inhaled for 2 hr through a one-way breathing valve by unanesthetized rats of 325-275 g. Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected during and following PCE inhalation and analyzed by gas chromatography. PCE exhaled breath and alveolar levels increased rapidly after the initiation of exposure to near steady-state within about 60 min. They were then directly proportional to the exposure concentration. Uptake of PCE in the blood was also rapid, but blood levels continued to increase progressively over the course of the 2-hr exposure at both dose levels. Cumulative uptake, or total absorbed dose, was proportional to the inhalation exposure level.

A blood flow limited physiologically-based model was characterized with PCE eliminated in the exhaled breath and to a limited extent by liver metabolism. PCE concentrations in the blood and exhaled breath were well predicted by the

PBPK model. The usefulness of model simulations in predicting systemically absorbed doses of PCE was demonstrated, which can have utility in risk assessments involving the internal dose of volatile organics.

VII. ESTABLISHING TET PARTITION COEFFICIENT PARAMETERS FOR PBPK MODEL IN DOGS

Physiologically-based pharmacokinetic (PBPK) models are a logical approach to improving the scientific basis for interspecies extrapolation in risk assessments of halocarbons and other volatile organic chemicals. In order to improve the accuracy of the model, predictions, tissue:blood partition coefficients were estimated using direct measurements of chemical TET in dog tissues and blood.

Animals were trained to stay calm and steady during experimentation, by getting them accustomed to sling, over a week's period. Dogs (6-9 kg) were utilized in these studies. For the animals intended to receive IA administration of the test compound, an indwelling jugular vein cannula was surgically implanted the day prior to the exposure. For procuring blood samples following TET administration, an indwelling carotid arterial cannula was implanted in the dogs. Both cannulas exited the body of the test animals behind the head, and the animals were allowed to recover from anesthesia until the following day. Food was withheld during the 18 hr recovery period before dosing.

The eight dogs were administered a loading dose of 5 mg/kg by jugular vein cannula over 1 min period, simultaneously infusion dose, at a rate of 28 μg/min kg, was started through the jugular vein. Both loading and infusion doses used polyethylene glycol (PEG) as a dosage vehicle. Infusion was for 4 hours. The IV administration was done u;sing 1 ml gas tight Hamilton syringe. Serial 20 μl blood samples were taken at selected intervals from 2 min to 4 hours following dosing. The concentrations of TET in the blood samples were determined by headspace analysis using a Perkin-Elmer Sigma 300 gas chromatograph. At the end of 4 hours, the dogs were immediately euthanized by using pentobarbital and saturated potassium chloride, and sacrificed. Approximately 1 g tissues of liver, kidney, fat, heart, lung, muscle, and brain were collected and transferred into 20 ml chilled scintillation vials containing 2 ml of saline and 8 ml of isooctane. Tissues were homogenized, vortexed and centrifuged as before. Twenty ml of the organic phase was withdrawn with a pipet and transferred to 8 ml headspace vials to be analyzed in the Perkin-Elmer sigma 300 gas chromatograph. The highest peak of TET concentration appeared immediately following loading dose.

The steady state was attained at about 45 min after dosing (Fig. C-1). The concentration at steady state was 0.750 μg/ml $\pm$ 0.07 which was close to projected concentration 1 μg/ml. The concentrations of blood and other tissues, at the end of 4 hours, are shown in table C-1. The result shown that highest concentration of TET was seen in fat tissue. In decreasing order were kidney, heart, brain, liver, lung and muscles. These parameters will be used for establishing tissue-blood partition coefficients of TET in dog which provide valuable input for halocarbon PBPK model parameter estimates.

VIII. PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL DEVELOPMENT WITH PHYSIOLOGICAL PARAMETERS FOR DOGS IN PBPK MODELS

When an investigator is attempting to gather physiological parameters as input functions in pharmacokinetic models, he often finds a wide gulf in literature, regarding this vital aspect. An attempt was made to rectify this deficiency, so that future modellers will have a ready source of information in reviewing the available data. Attention has been given to the sources where, experimental conditions were totally artificial, contrary to normal physiologic function. The breed, the weight range and the sex of the animals was identified for these studies. The literature was searched and primarily those data that are relevant to our purpose have been included. This selection was based primarily on the weight of the dog and sex of the dog. As much as possible, effort has been made to list parameters with a fair amount of uniformity in reporting.

Some methods described here are classic approaches. Most of the studies for measuring tissue blood flow employed microsphere techniques with various radioactive nuclides. Other methods included Wetterer, electromagnetic flowmeter, Fick method and dye dilution. Some researchers have utilized ^{39}Fe to measure erythrocyte mass, ^{131}I to measure plasma volume for determining liver blood flow. Doppler flow probe is one of the latest devices used in measuring blood flow measurement. Carbonized plastic radioactive microspheres have been used for perfusion of muscle. Measurements of blood flow to fat is one of the most difficult procedures. People have attemped to use various methods, such as silicone oil drop chamber and ^{133}Xe-wash-out. Some of the blood flow measurements have used constant perfusion rates. This condition seems very artificial and may not be useful in predicting accurate blood flow to the adipose tissue. Howewer, some researchers have used a somewhat physiological perfusion criterion, wherein the blood flow was maintained at 125 mm Hg which is closer to mean arterial pressure. So while compiling this kind of data from literature, it is imperative that thought be directed to the methods used in research and their applicability for a modeller in predicting/ assessing the physiologic parameter. Newer and more sophisticated methods have been used for measuring blood flow which probably are more accurate. Irrespective of the method, used, the investigator is obligated to use the value, which most approximates his experimental setup, in order to get a better fit of the model, since, any small change in the input parameter may have a significant impact on the model prediction.

Method of Measurement:

Direct Fick: The concentration of oxygen in the venous and arterial bloos are measured chemically, and rate of oxygen adsorption by the lungs is measeured by a respirometer through which the person breathes. Cardiac output can be calculated by comparing oxygen absorbed per minute by the lung with arteriovenous oxygen difference.

Dye-Dilution: A small amount of dye (i.e. Cardio-Green dye) is injected into a large vein, this then passes rappidly through the right heart, the lungs, the left heart, and finally into the arterial system. The concentration and duration of dye are recorded as it passes through one of the peripheral arteries for calculating cardiac output.

Electromagnetic flowmeter: Since blood is a conductor of electricity, the flow of blood with the vessel placed in a magnetic field genarates an electric potential across the vessel in accordance with the principle of electromagnetic induction. The electromagnetic flowmeter has a frequecy response more than adequate for the pulsatile wave forms found in the circulation. Its response is lenearly proportional to the velocity of blood flow; calibration either in vivo or in vitro is necessary to convert its electric output into adsolute units of velocity or volume flow per unit time.

Doppler Flow Probe: Transcutaneous Doppler flow probe reflect transit time of ultrasound wave from the moving blood to measure blood flow indirectly. The vessel is enclosed within the two halves of a cylinder with a crystal at each end, on opposite sides. These crystals act alternately as senders and receivers of a sound burst that passes diagonally across the vessel. The transit time downstream is shorter than upstream; from the difference between these electronically determined times, the volume flow through the vessel can be calculated.

Radioactive Microspheres: Regional blood flow were determined with microspere $1.0\text{-}1.5\text{x}10^6$ NEN-TRAC microspheres with a normal diameter of 15μm were used, labeled with one of the following radionuclides: ^{46}Sc, ^{113}Sn and ^{153}Cd, which were injected into left atrial catheter. After the animals were killed, organs and tissues were cut into small pieces and counted, allowing determination of the blood flow corresponding to each isotope. Counting was performed in a three-channel Auto-gamma Packard with appropriate settings for each isotope.

Erythrocyte Mass ^{59}Fe & Plasma volume ^{131}I: The dog received an infusion of freshly drawn compatible dog red cell tagged with ^{55} Fe for determination of circulating red cell volume. At the same time, an infusion of bovine albumin of fresh dog plasma iodinated with radioactive iodine (^{131}I) was given intravenously. The unit quantity of total red cells was based upon the relative concentration of hemoglobin in whole blood and that obtained by extraction of hemoglobin from tissue samples. The unit quantity of cell sample drawn just prior to death, and the tissue. Rapidly circulating red cell content was based on ^{59}Fe measurements. Total plasma and red cell content was taken as the product of unit content and organ weight. Iodine measurements reflect only circulating plasma.

Silicone Oil Drop Chamber: Drop recording of blood flow is that the blood drops collect and fall through a colorless incompressible silicone fluid. Silicone is fully indefferent relative to blood, it can be contacted with blood directely. the blood flow was measured by cannulating the artery leading to the adipose tissue and directing the blood by plastic tube to a photoelectric drop (silicone oil) recorder, which in turn operates an ordinate recorder.

^{131}Xe- Wash-Out Method: Xenon, due to its high lipid solubility, can be assumed to cross the lipidcontaining cellular membrane freely. ^{133}Xenon dissolved in sterile isotonic saline solution was injected into subjects. The disappearance rate of the isotope was measured with a sodium-iodide crystal, local clearance of ^{133}Xe has been applied to blood flow measurement.

Carbonized Plastic radioactive microspheres: Carbonized plastic microsphere, 9±1 μm in diameter, were labeled with gamma-emitting nuclides ^{141}Ce

(specific activity 12.4 m Ci/g) or ^{85}Sr (specific activity 12.86 m Ci/g). The microspheres were suspended in 10% low molecular weight dextran solution to which one drop of Tween-80 had been added to minimize aggregation. After 30 min of mixing, three to four million microspheres (0.5-1.0 ml) were injected into the left atrium. All tissues were cut in 1-3 g samples, the activity of each nuclide was determined with a three-channel gamma-well counter. Raw counts were then corrected and compared with the arterial, reference blood sample to obtain blood flow expressed in mililiters per minute per gram.

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IX. DETERMINATION OF HALOCARBON PARTITION COEFFICIENTS FROM TISSUE CONCENTRATION-TIME DATA

Tissue and blood concentration-time data from intraarterial administration of PCE in male Sprague-Dawley rats was utilized to obtain tissue-to-blood partition coefficients and to compare to model predicted PCE concentrations. Blood flows and tissue volumes were obtained from literature values. A blood-flow limited model was utilized with PCE eliminated unchanged in the exhaled breath and by saturable liver metabolism. The model consisted of tissue compartments for liver, kidney, fat, heart, lung, muscle, brain, blood and the rest of the body. Overall, there was good agreement between predictions of PCE tissue concentrations over time by the PBPK model and the direct measurements of PCE in rat tissues. Relative to other tissues, PCE muscle concentrations were initially overpredicted. Tissue area-under-the-concentration-time curves (AUCs) were predicted well with the PBPK model. In addition to their utility in developing PBPK model parameter estimates, tissue concentration time data were demonstrated to be useful in the validation of specific tissue compartments of a PBPK model for PCE by ia administration (See Figs D-8 through D-20).

These results were presented at the most recent national meeting of the Society of Toxicology in Seattle, Washingtonn. The reference for the abstract is as follows:

> Dallas, C.E., Varkonyi, P., Chen, X.M., Muralidhara, S., and Gallo, J.M. "Validation of a physiologically based pharmacokinetic model of perchloroethylene in rats using tissue concentration-time data." 31st Annual Meeting of the Society of Toxicology, Seattle, WA; Toxicologist 12: 346, 1992.

Male Sprague-Dawley rats (325-375 g), obtained from Charles River Laboratories, were employed in these studies. For the animals intended to receive the ia dose, an indwelling carotid arterial cannula was surgically implanted the day before the exposure. The cannula exited the body of the test animal behind the head, and the animals were allowed to recover from anesthesia until the following day. For ia administration, PCE was administered at a dose of 10 mg/kg as an emulsion in polyethylene glycol (PEG) in a single bolus dose through the carotid arterial cannula.

Groups of animals (n=4) were serially sacrificed (using etherization) following ia dosing at the following time intervals = 5, 10, 15, 30, and 60 minutes, and 1, 2, 4, 6, 12, 24, and 36 hours. Blood samples were obtained by cardiac puncture. Approximately 1 gram each of liver, kidney, brain, lungs,

heart, fat, and muscle were quickly removed and placed in 4 ml cold saline. Tissues were homogenized for the shortest possible time intervals, specific for each organ, to reduce the volatilization of the test compound during homogenization. The PCE in each sample was then extracted with 8 ml isooctane. A 20 μl aliquot was placed in an 8 ml headspace vial, which was capped and subjected to controlled temperature and pressure conditions in a Perkin-Elmer HS-6 Headspace Sampler.

Analysis was made of the PCE in the vial headspace on an OV-17 (6' x 1/8") stainless steel column in a Perkin Elmer gas chromatograph with an electron capture detector. The column temperatures were: detector-360°C, column-150°C, headspace-140°C, injector-200°C. Values were compared to a standard curve, and the tissue concentration corrected for the percent recovery characteristic for each tissue.

Due to the highly lipophilic nature of PCE, the maximum tissue concentration (C_{max}) in the fat was substantially higher (ranging from 1.6 to 11.4 times greater) than the other tissues. The tissue with the lowest rate of blood perfusion of those sampled, muscle, had the lowest C_{max}. The relative importance of perfusion in the tissue pharmacokinetics of PCE provided additional verification to the perfusion-limited approach to a PBPK model for PCE.

The area under the tissue concentration-time curve (AUC) for fat tissues was between 34 and 67 times greater than the AUC for liver, kidney, heart, lung, muscle, and brain. The tissue AUCs for liver, kidney, and brain were very similar, reflecting their similarity in a high rate of blood perfusion. The predictions of tissue AUCs for PCE were generally within 5% of the AUCs calculated from the observed tissue concentrations over time, except for liver and fat which had predicted tissue AUCs within approximately 9% of the observed tissue AUC.

In comparisons with observed tissue concentration-time data, tissue concentrations of PCE were well predicted by the PBPK model in brain, blood, kidney, heart, and lung over the length of the time course following PCE administration. In the first minutes following PCE exposure fat tissue levels were underpredicted, followed by fairly accurate predictions up to 84 hours, and terminal time points that were overpredicted relative to the observed fat PCE concentration. There was an initial over prediction of PCE concentrations in the muscle tissues up to 3 hours following administration, with more accurate predictions thereafter.

The PBPK model for PCE was therefore demonstrated to have considerable utility in accurately predicting tissue levels in rats. The least accurate predictions occurred in the most poorly perfused tissue sampled, muscle, and in the fat tissues more than 3 days following exposure. Tissue AUC proved to be a parameter accurately predicted by the PBPK model, which may be of significant utility in defining the applicability of this and similar models in risk assessments pertaining to specific compartments of the model.

X. TISSUE TIME COURSE KINETIC STUDIES OF PCE IN RATS FOR CORRELATION WITH NEUROBEHAVIORAL MEASUREMENTS

Exposures to volatile organic compound (VOCs) result from their widespread commercial use and improper disposal. VOC exposures may produce central nervuos system (CNS) depressant effects. The intensity and time course of these CNS effects are assumed to be dependent upon the level of VOC in the brain. The relationship between brain dose and neurobehavioral response must be inferred from animal studies. PBPK models were developed to describe a chemical's dynamics in the blood and specific organs. Furthermore, chemical's effects are being quantified and used to establish biological repsonse or toxicodynamic models. Once the relationship between brain concentration and neurobehavioral toxicity is established a toxicodynamic model can be developed to predict human neurobehavioral effects for a given chemical exposure.

Male Sprague-Dawley rats (300-350 g) were maintained on a restricted diet (10 g/day) for 72 hours prior to being surgically implanted with an indwelling carotid artery cannula. After an overnight recovery period, rats received 1 ml of oral bolus of either 160 or 480 mg/kg PCE in a 10% aqueous Emulphor® emulsion. Serial blood samples were collected via the carotid artery cannula at 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, and 90 min upto 72 hours (Fig. D 1-3).

Male Sprague-Dawley rats (300-350 g) were maintained on a restricted diet (10 g/day) for 72 hours prior to receiving a 1 ml oral bolus of either 160 mg/kg PCE in a 10% aqueous Emulphor® emulsion or 480 mg/kg PCE in a 20% aqueous Emulphor® emulsion. Animals were serially sacrificed by decapitation at 1, 6, 12, 15, 20, 30, 40, 50, 60, and 90 minutes post dosing. Samples of brain, liver, fat and muscle were removed immediately and placed in chilled scintillation vials containing 2 ml of saline and 8 ml of isooctane. Tissues were homogenized, vortexed, and centrifuged. Blood samples and aliquots of the tissue supernatant were dilated in saline and placed in 20 ml headspace vial analyzing in Perkin-Elmer Model 8500 gas chromatograph.

Absorption of PCE following oral administration was relatively slow in rats, with peak blood concentration of 45 min and 105 min following 160 and 480 mg/kg dosing respectively (Fig. D-1, 2, 3; Table D-1,2). The maximum concentration of PCE in the blood for 160 mg/kg dose was 24.17 μg/ml; and at 480 ml/kg dose was 89 μg/ml. The half-life of PCE in both doses were relatively close, 8.4 hrs and 9.25 hrs respectively. Following oral administraiton of PCE, AUC of 480 mg/kg dose was 2.65 times as high as AUC of 160 mg/kg dose.

In the tissue studies of both 160 mg/kg and 480 mg/kg upto 90 min (Fig. D-4.5), all tissues show steady state levels from 15 min onward until termination. The AUC for fat was highest, and in the following order were liver, brain, muscle, and blood. The result shows that brain concentrations were lower than in fat and liver.

XI. NEUROBEHAVIORAL MEASUREMENTS IN RATS DURING AND FOLLOWING PCE AND TRI EXPOSURE

One objective of the project is to develop a combined physiological pharmacokinetic-toxicodynamic model that would allow the prediction of behavioral toxicity given brain concentrations derived from a validated physiologically-based pharmacokinetic model. To this end, we are currently attempting to define the relationship between the brain concentration of a particular halocarbon and the resulting behavioral toxicity. Thus far, our efforts have been concentrated on halocarbon-induced behavioral impairment in rats as measured in various operant paradigms. Halocarbons have been administered by oral gavage as well as by inhalation.

A dose-range finding study was conducted in which male Sprague-Dawley rats were administered Perchloroethylene (PCE) as a single oral bolus, after which their behavior was evaluated under a fixed-ratio 40 schedule of reinforcement for 90 minutes. To a lesser extent, the study evaluated the effect of various dosing vehicles and training time on PCE-influenced behavior. Additionally, the concentration of PCE in blood, brain, liver, fat and muscle was determined for up to 90 minutes following oral dosing with either 160 or 480 mg/kg PCE. The study's results were presented in part in poster form at the latest meeting of the Society of Toxicology that was held in Seattle, Washington in February 1992. The poster is found in its entirety as Appendix E-1 to E-13. The reference for this abstract is as follows:

> Warren, D.A., Dallas, C.E., Reigle, T.G., and Muralidhara, S.: "Neurobehavioral Toxicity of Ingested Perchloroethylene in Rats." 31st Annual Meeting of the Society of Toxicology, Seattle, Washington: The Toxicologist: Vol.12, No.1 (1992).

Based upon the results of the above study, a larger study was conducted in which male Sprague-Dawley rats were administered a single oral bolus of either 160 or 480 mg/kg PCE, after which their behavior was evaluated under a fixed-ratio 40 schedule of reinforcement for 90 minutes. This study resulted in a behavioral data set that can be interpreted with full knowledge of blood and brain levels of PCE at various times during the operant session. Although the interpretive phase of the study is not yet complete, it is clear that the operant paradigm employed was sensitive to the animals' dose-dependent behavior. It is apparent from Figures E-14 and E-15 that the administration of a vehicle placebo did not result in deviations from normal behavior beyond that expected from inter-day variability in the animals' response rates. Likewise, Figure E-16 indicates that a dose of 160 mg/kg PCE did not result in behavioral depression, whereas Figure E-17 implicates 480 mg/kg as a behaviorally depressive dose of PCE from which recovery was gradual as the operant session progressed.

In another study, an operant test cage was placed inside an inhalation chamber so that behavior could be monitored concurrent with exposure. The study was undertaken to examine the feasibility of cumulative dosing by inhalation. The use of cumulative dosing would be of great benefit in establishing dose-response relationships due to the time that must be expended to shape and train experimental animals. Male Sprague-Dawley rats were allowed to establish a baseline response rate for 40 minutes under a variable interval-60 schedule of

reinforcement. After 40 minutes, the animals were exposed to either 1000, 2000, 3000, 4000, or 5000 parts per million Trichloroethane (TRI) for 100 minutes. Forty-eight hours after exposure, the absence of any residual behavioral effect was verified. Seventy-two hours after exposure, each animal was exposed to a different concentration of TRI, until each animal had been exposed to all five concentrations. A cursory look at the data reveals that cumulative dosing with TRI by inhalation may not be feasible, as one TRI exposure may influence behavior during subsequent exposures. This appears to be the case even though bioaccumulation of TRI does not occur under the exposure schedule employed. Sudden deviations from linearity upon initiation of exposure indicates that the animals may be sensitized to the odor and/or irritant properties of TRI (Figure E-18). Defining an accurate dose-response relationship using cumulative dosing by inhalation may also be hindered by inter-day variability in response rates.

As an alternative to cumulative dosing, a study is underway in which animals are exposed to only a single concentration of TRI. Male Sprague-Dawley rats are exposed to a single concentration of TRI from 1000 to 5000 parts per million by inhalation, during which time behavior is measured under a variable-interval 30 schedule of reinforcement. As expected, rats maintain a stable rate of baseline responding over the duration of the operant session resulting in a linear, and thus predictable, cumulative response curve (Figure E-19). We believe that a quantitative measure of halocarbon-induced behavioral impairment may be obtained by measuring an animals' deviation from linearity under exposure conditions. As seen from the representative data in Figures E-20 through E-24, the magnitude of deviation from linearity occurs in a dose-dependent manner. Upon completion of the behavioral study, the uptake of TRI into tissues and blood will be measured during exposures to concentrations equivalent to those employed in the behavioral study. If the magnitude of deviation from linearity is found to correlate with the brain concentration of TRI, this would represent major progress toward the development of a toxicodynamic model predictive of halocarbon-induced behavioral changes. It is expected that preliminary data from this study will be presented at the next Annual Meeting of the Society of Toxicology in New Orleans, La.

XII. COLLABORATIVE ARRANGEMENTS

The proposed project has been conducted at the Department of Pharmacology and Toxicology (P & Tx) and the Department of Pharmaceutics in the University of Georgia (UGA) College of Pharmacy. The Principal Investigator has been Dr. Cham E. Dallas (CED), who has been responsible for overall coordination of the project. He has provided a 20% commitment to this project. In addition to coordinating the project, CED personally conducted all of the respiratory elimination studies that were done. In that effort he developed the exposure system for the direct kinetic determinations of halocarbons, along with monitoring of respiratory parameters. Under CED's direction, the assay was developed for measuring halocarbon levels in the tissues of animals following exposure, which has been of significant utility in the present investigation. A primary focus of CED's studies has been to provide data sets for the development and validation of physiologically-based pharmacokinetic (PBPK) models. Dr. James V. Bruckner (JVB) has provided a 10% commitment to the project as a Co-Principal Investigator. He has directed a number of research projects on the oral toxicity and pharmacokinetics of volatile organic compounds over the

past 10 years. JVB is also experienced in the application of pharmacokinetic data to risk assessments, having served on a number of committees and advisory groups for federal agencies concerned with health effects of VOCs. Dr. James M. Gallo (JMG) has served as a Co-Investigator (5% commitment), and provided expertise in the field of pharmacokinetics (PK). The major focus of his work has been the physiologically-based pharmacokinetic (PBPK) modeling, including the derivation of methods for estimation of mass transfer coefficients and partition coefficients for PBPK models. JMG had primary responsibility for design of PK studies and analysis of data, development and refinement of PBPK models, and assessment of the model's ability to predict halocarbon disposition in humans. Dr. Randall Tackett (RT) served as a Co-Investigator on the project (10% commitment), and was responsible for the kinetic experiments in the dog in this project. RT heads an active laboratory staffed by postdoctoral associates and graduate students, in which a number of toxicodynamic studies in the dog and the rat have been conducted. Dr. Tom Reigle (TR) also served as a Co-Investigator on the project (20% commitment). TR provided valuable assistance in the selection and purchase of the appropriate testing equipment that can be used for both rats and dogs, and was involved in the design and conduct of all the neurobehavioral studies.

Mr. Alan Warren (AW) is a doctoral student who has been conducting his dissertation research involving the research objectives of this grant. Alan is the recipient of a three year award from the Department of Defense, managed by the Southeastern Center for Electrical Engineering Education (SCEEE). This award provides for his graduate assistantship stipend and approximately $2000 annually for travel and minor expenses. As the SCEEE award coincides almost exactly with the period of this Air Force grant, this is an important (and much appreciated) collaborative effort. AW was in charge of the conduct of all of the neurobehavioral studies conducted during the first year of the grant. He has personally been involved in the development of the neurobehavioral testing protocols, and has provided a very perseverant effort toward the success of this critical part of the project. He has been assisted by Warren Christmus, a Pharmacy student who has worked approximately half-time over the year on the neurobehavioral training. Mr. Li You, a doctoral student, worked on the analytical measurements of halocarbon concentrations in animal tissues, and technical and clerical assistance was also provided by Wade Meredith, a masters student in the Department of Pharmacology and Toxicology.

Dr. Xiao Mei Chen (XMC) has served as a full-time postdoctoral associate on the project. XMC has a medical degree from the People's Republic of China, and has worked in the current Air Force project since its inception. She was successful in her work in the development of the assay for the measurement of halocarbons in the tissues of exposed animal and has conducted these tissue measurements thus far for ia and po exposures for PER and TET in rats and dogs. Dr. Peter Varkonyi (PV) is a postdoctoral associate from Hungary who has worked "hands-on" with the development of the PBPK models for halocarbon pharmacokinetics. Mr. Srinvasa Muralidhara has been employed part-time (25%) on the project. He participated in the analysis of halocarbons in biological samples, computer programming, the conduct of inhalation studies of dynamic exposure chambers, and the compiling of laboratory records.

XIII. INTERACTION WITH DOD LABORATORIES

There is currently much debate among behavioral toxicologists as to the interpretation of neurobehavioral data and the appropriateness of specific methods used to collect it. In an effort to participate in this scientific debate and stay abreast of developments in the field of neurobehavioral toxicology, Dr. Cham Dallas and Mr. Alan Warren attended a conference entitled, "Toxicological Interpretation of Neurobehavioral Data." The conference was held at the University of Rochester Medical Center, Rochester, NY., on July 22-24, 1992. Attendance at this conference provided the opportunity to solicit the opinions of prominent behavioral toxicologists from academia, government, and the private sector. The program from the Rochester conference is included as E-25 through E-30. Additionally, Mr. Warren recently visited the toxicology division at Wright-Patterson Air Force Base (WPAFB), an opportunity afforded him as a recipient of a Department of Defense Science and Engineering Fellowship. While at WPAFB, Mr. Warren met with his Air Force Laboratory mentor, Dr. Jeff Fisher, as well as other Air Force and contract scientists with expertise in pharmacokinetic modeling and toxicodynamics. Other opportunities to discuss neurobehavioral toxicology should arise at the next meeting of the Southeastern Society of Toxicology scheduled for October 15-16 in Athens, Ga. The meeting will include a symposium entitled "Neurotoxicology: Experimental, Clinical and Environmental Aspects." Lastly, Mr. Warren has arranged for Dr. Christopher Newland of Auburn University to deliver a seminar to the Department of Pharmacology and Toxicology, University of Georgia, on his use of schedule-controlled operant behavior in toxicological studies.

Cumulative List of Research Articles and Abstracts

on Research Completed in Year 1

Dallas, C.E., Ramanathan, R., Muralidhara, S., Gallo, J.M., and Bruckner, J.V. "Physiologically based modeling with inhaled trichloroethylene in rats." Toxicology and Applied Pharmacology 110: 303-314 (1991).

Chen, X.M., Dallas, C.E., Muralidhara, S., Srivatsan, V., and Bruckner, J.V. "Determination of volatile halogenated hydrocarbons in body tissues." Submitted to Journal of Chromatography (1992).

Warren, D.A., Dallas, C.E., Reigle, T.G., and Muralidhara, S. "Neurobehavioral toxicity of ingested perchloroethylene in rats." 31st Annual Meeting of the Society of Toxicology, Seattle, WA; Toxicologist 12: 276, 1992.

Dallas, C.E., Muralidhara, S., Ramanathan, R., Gallo, J.M., Varkonyi, P., and Bruckner, J.V. "Development of a physiologically based pharmacokinetic model for inhaled perchloroethylene in rats," now being submitted to Toxicology and Applied Pharmacology.

Muralidhara, S., Srivatsan, V., Sanzgiri, U., Dallas, C.E., and Bruckner, J.V. "Improvement of extraction efficiency in analysis of volatile organic compounds (VOCs) in tissues." 31st Annual Meeting of the Society of Toxicology, Seattle, WA; Toxicologist 12: 424, 1992.

Dallas, C.E., Varkonyi, P., Chen, X.M., Muralidhara, S., and Gallo, J.M. "Validation of a physiologically based pharmacokinetic model of perchloroethylene in rats using tissue concentration-time data." 31st Annual Meeting of the Society of Toxicology, Seattle, WA; Toxicologist 12: 346, 1992.

TISSUE VOLUMES OF DOG

Tissue	Value (range)	Breed	N	Sex	Age	bw (kg)	Reference
Liver	380 g	beagle				9	1 p.230
	530 g					12-19	2 p.283
	373 (250-450) g	beagle					1 p.85
	adult approx. 4 % of bw	beagle					1 p.85
	2.94 g/100 g		4	M F		13	2 p.163
Kidney	25 g/each (with both 0.5% of bw)	beagle				9-10	1 p.294
	40-60 g adult approx. 0.5% of bw	beagle			5 month		1 p.89
	0.3 g/100g		4	M F		13	2 p.163
	31 g/each 0.34% of bw.					9.1	2 p.174
Fat	1986 (960-3520)g 16.5% bw		5			11.6(8.8-13.8)	23 p.198
	20.1 (14.6,25.6)% of bw		2			6.04	24 p.515
	13.1 (9-26)% of bw	beagle	8	F2 M6		8.9(7.2-12.2)	25 p.1037
	14.9±1.13% of bw	beagle	18		3-13 months		26 p.139-146
	14.7±1.34% of bw	beagle	11		1-2 years		26 p.139-146
	2980±500 g 29% of bw	beagle		F	3 years	10.26±0.43	27 p.278-281
	3100±360 g 30% of bw	beagle		F	3 years	10.22±0.33	27 p.278-281
	3300±2200 (300-11100) g 26.3±9.3 (4.5-45.2)% of bw	beagle	24	F	2-4 years	11.1±3.3 (7.2-23.1)	28 p.367-370
	1033 (384-2424) g 15.2 (9.1-26)% of bw	beagle	11	F4 M7	88-185 days	6.8 (4.13- 12.16)	29 p.369-376)
Heart	81±15 (60-100) g 0.6-1.0% of bw 7.3 g/kg	beagle					1 p.86
	81.4 (46-129.2) g	beagle				7.18 (3.33- 12.05)	1 p.560
	1.13 g/100 g	beagle					1 p.560
	0.85 g/100 g		4	F M		13	2 p.163

Tissue	Valve	Breed	N	Sex	Age	bw (kg)	Reference
Lung	75±8 (41-104) g	beagle	20	M		11.5±1 (7.0-14.9)	1 p.287
	82±17 g; adult approx. 1% of bw	beagle	4	F M		13	1 p.87
	65-119 g	beagle	10	M			1 p.287
	6.5±0.4 g/kg	beagle		M		11.5±1 (7.0-14.9)	1 p.287
	6.15-8.49 g/kg	beagle	10	M			1 p.287
	8.5±1.5 g/kg	beagle	20	M		11±2.1	1 p.287
	0.94 g/100 g		4	F M		13	2 p.163
Muscle	4639 g; 49.2% of bw	beagle		M	81 days	9.4	1 p.83
	2188 g; 40.8% of bw	beagle		F	122 days	5.37	1 p.83
	3611 g; 46.8% of bw	beagle		F	236 days	7.711	1 p.83
	46 (35-54) % of bw	beagle	16	F M			1 p.83
Brain	60-80 (72±6) g adult approx. 0.5-1.0% of bw				>3 months		1 p.84
	0.59 g/100 g		4	F M		13	2 p.163
Blood	81.9 ml/kg	beagle	14	F M	4.5±0.7 years	10.86±0.2	1 p.278
	81.7 ml/kg	beagle			47 weeks		1 p.278
	94.1 (76.5-107.3) ml/kg		11				8 p.264
	35-40 ml/lb; 8-9% of bw						11 p.277
	7.7% of bw						13 p.282
	6-7% of bw in all animals						5

BLOOD FLOW DISTRIBUTION IN DOGS

Tissue	Value	Condition	Breed	N	Age Sex	bw (kg)	Method	Reference
Cardiac Output	1.3-3.0 L/min	Standing position Unanethetized	beagle			8-12		1 p.241
	1.12 (0.65-1.57) L/min	Basal (Morphine)				6.4	Direct Fick	2 p.279
	1.18 (0.8-1.59) L/min	basal (Narcosis)				11.8	Wetterer	2 p.279
	2.8±0.2 L/min			9		18-25	electromagnetic flowmeter	4 p.929
	2.5±0.3 L/min	Stood quietly		14		17-23	electromagnetic flowmeter	6 p.1515
	6.7±0.3 L/min		foxhound	9		22.8±0.7		7 p.1724
	2.21 (1.2-3.84) L/min	basal				16.1	Direct Fick	2 p.279
	3.9 (3.6-4.1) L/min	standing				21.5	Direct Fick	2 p.279
	135 ml/kg/min	37-39°C						2 p.438
	180 ml/kg/min	barbiturate anesthesia					Direct Fick	11 p.314
	1.82 (1.14-2.5) L/min	anesthesia				14.4	Stew. NaCl	2 p.279
Liver	82 (46-112) ml/min/100g	basal				12-19		2 p.283
	33.6±4 ml/min/100g 6.95±0.95% of CO	awake	mongrel	25	M	19.6±0.7	radioactive microspheres ^{46}Sc, ^{113}Sn, ^{153}Cd	31 p.H663-H669
	38±3 ml/min/100g	awake	mongrel	7		15-25	carbonized plastic microspheres ^{51}Cr, ^{125}I	32 p.109-121
	20±5 ml/min/100g	awake		10		18-27	radioactive microspheres ^{85}Sr, ^{141}Ce, ^{125}I, ^{46}Sc	33 p.377-382
	35±6 ml/min/100g	awake	mongrel	7		20-28	radioactive microspheres ^{51}Cr, ^{85}Sr, ^{46}Sc, ^{141}Ce	34 p.175-182
	25±7 ml/min/100g	awake	beagle	7	F.M	6.5-15.2	raddioactive microspheres ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{46}Sc	35 p.1321-1336
	44±4 ml/min/100g	awake	mongrel	8		15-25	carbonized platic radioactive microspheres ^{141}Ce, ^{85}Sr	36 p.1055-1061
	35±5 ml/min/100g	awake		14		20	radioactive microspheres ^{141}Ce, ^{85}Sr, ^{51}Cr	37 p.171-178
	21±11 ml/min/100g			14		17-23	radioactive microspheres ^{125}I, ^{141}Ce ^{85}Sr, ^{46}Sc	6 p.1517
	30±7 ml/min/100g		foxhound	9		22.8±0.7	radioactive microspheres ^{46}Sc, ^{85}Sr, ^{113}Sn, ^{125}I, ^{57}Co, ^{93}Nb	7 p.1728
	207±28 ml/min	awake	mongrel	25	M	19.6±0.7	radioactive microspheres	31 p.H663-H669
	387 (216-612) ml/min/total tissue 530 g					12-19		2 p.283
	10 ml/min/100 g			9		18-25	radioactive microspheres ^{85}Sr, ^{141}Ce, ^{51}Cr	4 p.927
	2.5% of CO (2.8 L/min)		mongrel	9		18-25	radioactive microspheres	4 p.927

Tissue	Value	Condition	Breed	N	Age Sex	bw (kg)	Method	Reference
Liver	147μl/g						Erythrocyte mass ^{59}Fe Plasma volume ^{131}I	9 p.848
	35-45 ml/min/kg 30 of CO						Fick & Dye-dilution	12 p.134
Kidney	169±11 ml/min	awake		6				3 p.391
	173±10 ml/min	awake		6				3 p.396
	207±15 ml/min	awake	beagle	16	adult	10.0	radioactive microspheres ^{85}Sr	38 p.229-240
	440±35 ml/min	awake	mongrel	25	M	19.6±0.7	radioactive microspheres	31 p.H663-H669
	145±5 ml/min	awake	mongrel	10		20-30	Doppler flow probe (in vivo)	39 p.829-836
	96±13 ml/min	awake		9				3 p.396
	158±10 ml/min	awake	mongrel	17	F.M	20-30	Doppler flow probe, radioactive microspheres & electromagnetic flowmeter	40 p.185-195
	417.5±22.1 ml/min/100g 14.61±0.76% of CO	awake	mongrel	25	M	19.6±0.7	radioactive microspheres	31 p.H663-H669
	377±56 ml/min/100g	awake	mongrel	7		15-25	radioactive microspheres	32 p.109-121
	545±53 ml/min/100g	awake	mongrel	7		20-28	radioactive microspheres	34 p.175-182
	377±49 ml/min/100g	awake	beagle	7	F.M	6.5-15.2	radioactive microspheres	35 p.1321-1336
	387±41 ml/min/100g	awake	mongrel	8		15-25	carbonized plastic radioactive microspheres	36 p.1055-1061
	365±61 ml/min/100g	awake		14		20	radioactive microspheres	37 p.171-178
	220 ml/min/100g		mongrel	9		18-25	radioactive mocrospheres	4 p.929
	10% of CO (2.8±0.2 L/min)		mongrel	9		18-25	radioactive microspheres	4 p.929
	347±54 ml/min/100g			14		17-23	radioactive microspheres	6 p.1517
	405±17 ml/min/100g		foxhound	9		22.8±0.7	radioactive microspheres	7 p.1728
	81 μl/g						Erythrocyte mass & plasma volume	9 p.848
C_{at}	16.7 (3.7-29.4) ml/min/100g	inguinal subcutaneous fat pad	mongrel	25	F M	14.9 (8.6-20)	silicone oil drop chamber	14 p.65-70
	14.6±4.4 ml/min/100g						radioactive microspheres ^{127}Xe, ^{133}Xe & washout method	15 p.471-4
	18.3±6.2 ml/min/100g	perirenal	mongrel		10 weeks	5	albumin microspheres labelled with ^{99}Tc or with ^{46}Sc	16 p.81-85
	2.89-5.87 ml/min/100g		mongrel	43		15-39	blood flow measurement at a constant rate	17 p.1327-33

Tissue	Value	Condition	Breed	N	Age Sex	bw (kg)	Reference
Liver	147μl/g						9 p.383
	35-45 ml/min/kg 30 of CO						12 p.134
Kidney	169±11 ml/min	awake		6		8-14	3 p.391
	173±10 ml/min	awake		6		8-14	3 p.396
	207±15 ml/min	awake					3 p.396
	440±35 ml/min	awake		19			3 p.396
	145±5 ml/min	awake	mongrel	10		22±3	3 p.396
	96±13 ml/min	awake	mongrel	9		20-30	3 p.396
	158±10 ml/min	awake		17			3 p.396
	417.5±22.1 ml/min/100g 14.61±0.76% of CO	awake		19			3 p.398
	377±56 ml/min/100g	awake		7			3 p.398
	545±53 ml/min/100g	awake		7			3 p.398
	377±49 ml/min/100g	awake		7			3 p.398
	387±41 ml/min/100g	awake		8			3 p.398
	365±61 ml/min/100g	awake		14		18-24	3 p.398
	220 ml/min/100g		mongrel	9		18-25	4 p.929
	10% of CO (2.8±0.2 L/min)		mongrel	9		18-25	4 p.929
	347±54 ml/min/100g			14		17-23	6 p.1517
	405±17 ml/min/100g		foxhound	9		22.8±0.7	7 p.1728
	81 μl/g						9 p.383
Fat	16.7 (3.7-29.4) ml/min/100g	inguinal subcutaneous fat pad	mongrel	25	F M	14.9 (8.6-20)	14 p.65-70
	14.6±4.4 ml/min/100g						15 p.471-4
	18.3±6.2 ml/min/100g	perirenal	mongrel		10 weeks	5	16 p.81-85
	2.89-5.87 ml/min/100g		mongrel	43		15-39	17 p.1327-33

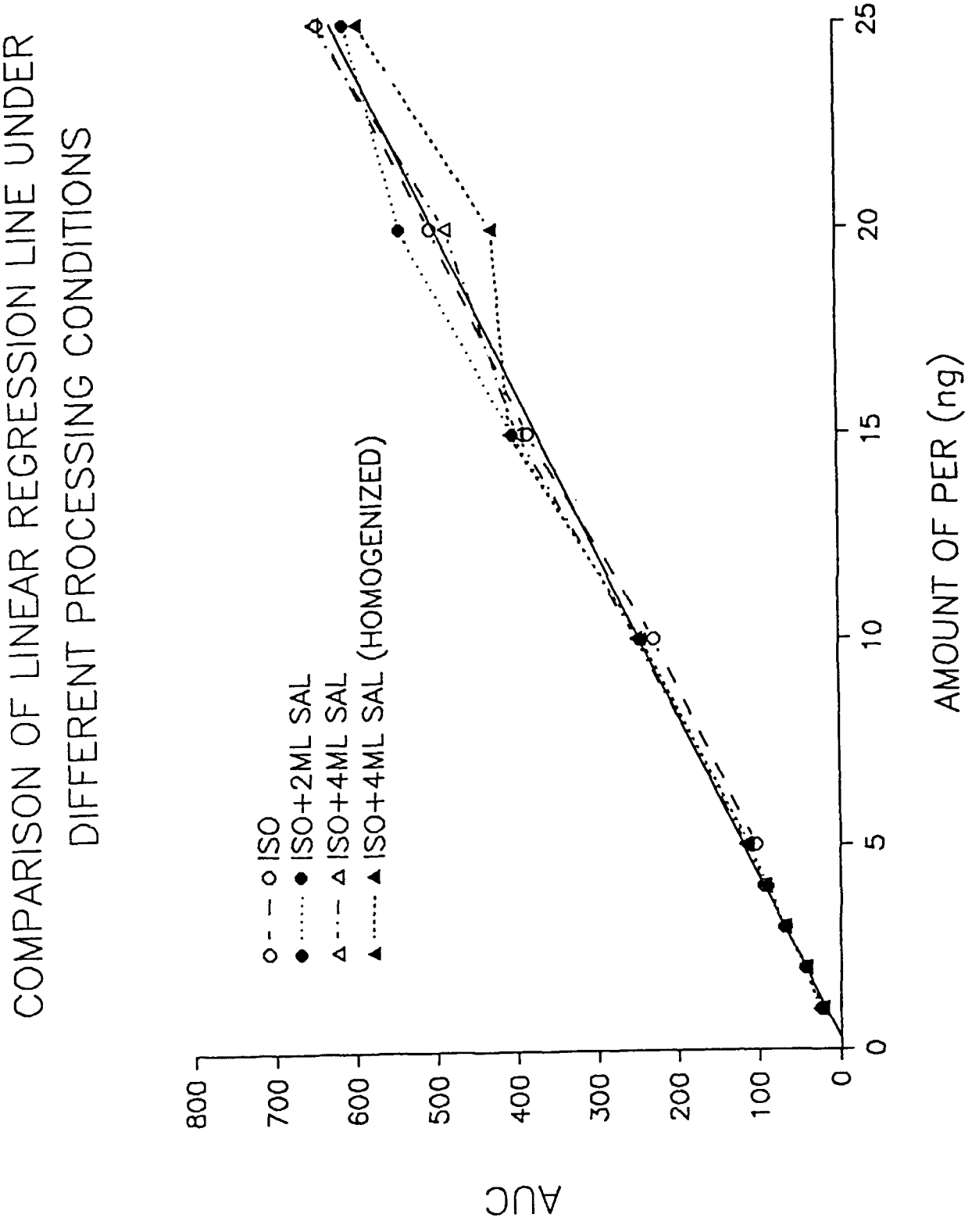
Tissue	Value	Condition	Breed	N	Age	bw (kg)	Reference
Fat	3.9 (1.9-11) ml/min		mongrel beagle	7	F	12-15	18 p.169-174
	8.5 (3-19) ml/min/100g	subcutaneous	mongrel	17	F	8-14	19 p.397-403
	2.2-30 ml/min/100g	subcutaneous	mongrel	17	F	22.8±0.7 (17.9-28.9)	20 p.932-938
	6.4 ml/min/100g	omental	mongrel	11	M	12.4	21 p.367-74
	5.5 ml/min/100g	subcutaneous	mongrel	11	M	12.4	21 p.367-74
Heart	70.1 (52-103) ml/min 54% of CO					9-19	2
	77±10 ml/min/100g	resting		14		17-23	6 p.1517
	133 (79-220) ml/min/100g	basal				9-10	2 p.283
	66 µl/g						9 p.383
Lung	2300-3200 ml/min						2
	123±14 ml/min	awake					3 p.397
	40.1±4.4 ml/min/100g 4.07±0.4% of CO	awake		19			3 p.399
	24±3 ml/min/100g	awake		7			3 p.399
	54.8±8 ml/min/100g	awake		7			3 p.399
	301 µl/g						9 p.383
Muscle	25.9±2% of CO	awake		19			3 p.400
	22±3 ml/min/100g	awake		7			3 p.400
	3.7±0.6 ml/min/100g	awake		7			3 p.400
	3±1 ml/min/100g	awake		7			3 p.400
	24±3 ml/min/100g	awake		8			3 p.400
	3±1 ml/min/100g	awake		14		20 (18-24)	3 p.400
	15±4 ml/min/100g	intercostal resting		14		17-23	6 p.1517

Tissue	Value	Condition	Breed	N	Age	bw (kg)	Reference
Muscle	11 μl/g						9 p.383
Brain	64±5 ml/min	awake					3 p.397
	64±3 ml/min	awake		19			3 p.397
	68.1±3.3 ml/min/100g 2.2±0.1% of CO	awake		19			3 p.399
	80±9 ml/min/100g	awake		7			3 p.399
	55±4 ml/min/100g	awake		7			3 p.399
	61±6 ml/min/100g	awake		7			3 p.399
	80±9 ml/min/100g	awake		8			3 p.399
	38±4 ml/min/100g	awake		14		18-24	3 p.399
	62±3 ml/min/100g	resting		14		17-23	6 p.1517
	76±4 ml/min/100g		foxhound	9	M	22.8±0.7	7p.1728
	11 μl/g						9 p.383

DATA OF ALVEOLAR VENTILATION

Respiratory Rate Breath/min	Tidal Volume ml	Minute Volume L/min	Alveolar Ventilation %/VE	Breed	N	Age	Sex	BW (kg)	Method	Reference
18 (11-37)	320 (251-432)	5.21(3.3-7.4)						16.4-30.5		8 p.220
18 (11-38)	320(251-432)	5.2(3.3-7.4)								2 p.267
30	69±38	1.8±0.7	68±28 Ve	beagle	10	3 m				10 p.173
21±6	178±58	3.6±0.9	71±7 Ve	beagle	140-240	1 y				10 p.173
15.5±12.38 11.23±8.02	198.88±81.64 206.77±121.06	2.9±2.3 1.8±1.2			42 35	6.38±4.87 5.09±5.09	M F	20.99±8.38 16.75±8.16	pneumotachograph	11 p.173-178
	175									13 p.347
23±2	204±20 (16±2 ml/kg)	4.5±0.2 (345±18 ml/kg)		beagle	20		M F	13.1±0.2	wet testmeter & thermister	1 p.292
	300.4±23.8 anesthetized			mongrel	8			20-30	pneumotachograph & differential pressure transducer	22 p.1060-7

Fig. A-1


COMPARISON OF LINEAR REGRESSION LINE UNDER
DIFFERENT PROCESSING CONDITIONS
ISO
ISO+2ML SAL
ISO+4ML SAL
ISO+4ML SAL (HOMOGENIZED)
AUC
AMOUNT OF PER (ng)
0
100
200
300
400
500
600
700
800
0
5
10
15
20
25

EFFECT OF THERMOSTATTING TIME ON HEADSPACE EQUILIBRIUM

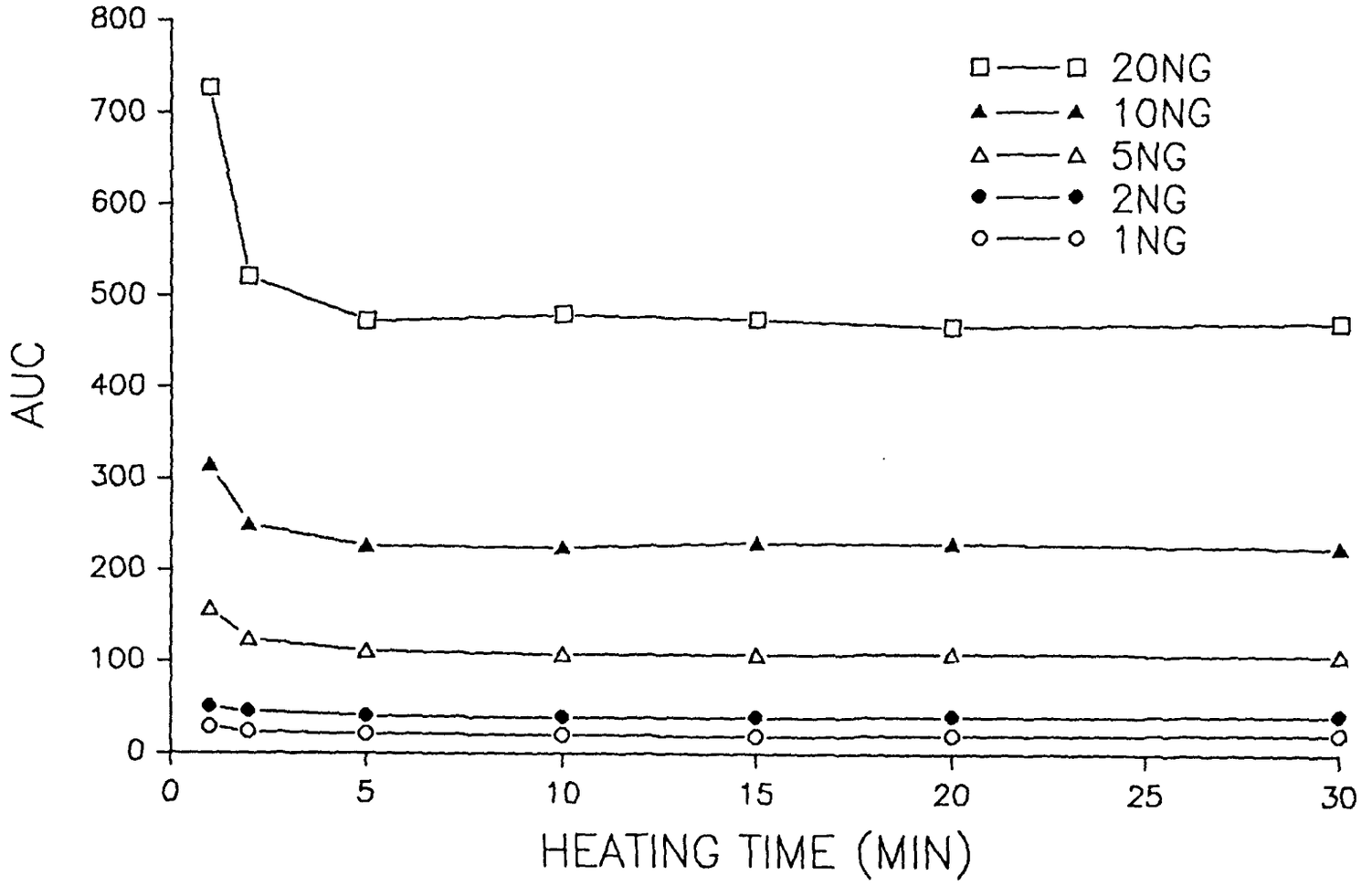


Fig. A-2

PERCENT RECOVERY OF PCE IN RAT TISSUE
AT FOUR CONCENTRATIONS

	0.1 mg/ml	1 mg/ml	5 mg/ml	20 mg/ml	MEAN	SD	SE
LIVER	85.67	101.83	105.83	94.67	97.00	8.85	4.43
KIDNEY	91.00	97.50	99.50	93.67	95.42	3.81	1.91
FAT	84.17	91.33	98.00	95.50	92.25	6.05	3.02
HEART	91.67	99.83	96.33	96.83	96.17	3.37	1.69
LUNG	86.50	89.83	102.00	92.17	92.63	6.67	3.33
MUSCLE	92.00	94.00	95.50	93.50	93.75	1.44	0.72
BRAIN	89.17	92.83	97.33	90.83	92.54	3.53	1.76
BLOOD	92.33	96.83	103.00	97.83	97.50	4.38	2.19

PERCENT RECOVERY OF PCE IN TISSUE
AT SIX CONCENTRATIONS

	0.1 mg/ml	0.5 mg/ml	1 mg/ml	5 mg/ml	10 mg/ml	20 mg/ml	MEAN	SD	SE
LIVER	96.5	92.8	95.3	95.7	91.0	97.5	94.8	2.4	1.0
KIDNEY	98.0	95.2	95.0	97.5	94.2	99.8	96.6	2.2	0.9
FAT	103.2	88.3	92.5	94.3	90.3	92.3	93.5	5.2	2.1
HEART	97.8	96.0	91.0	92.3	92.7	96.7	94.4	2.8	1.1
LUNG	97.8	90.2	98.0	96.2	94.5	95.0	95.3	2.9	1.2
MUSCLE	98.0	94.3	92.2	95.8	91.3	94.7	94.4	2.4	1.0
BRAIN	99.0	92.3	92.7	94.8	93.7	93.5	94.3	2.4	1.0
BLOOD	92.7	96.7	96.2	101.5	102.2	97.2	97.8	3.6	1.5

Table B-1

PERCENT RECOVERY OF TET IN RAT TISSUE
AT FOUR CONCENTRATIONS

	0.1 mg/ml	1 mg/ml	5 mg/ml	20 mg/ml	MEAN	SD	SE
LIVER	82.7	98.2	89.7	96.2	91.70	7.01	3.51
KIDNEY	81.0	93.5	90.0	87.7	88.05	5.27	2.64
FAT	85.7	91.0	86.8	89.7	88.30	2.47	1.23
HEART	97.2	90.3	95.5	91.7	93.68	3.22	1.61
LUNG	90.7	90.8	87.3	99.7	92.13	5.31	2.65
MUSCLE	86.5	92.8	87.5	84.3	87.78	3.61	1.80
BRAIN	93.3	89.0	83.5	92.0	89.45	4.36	2.18
BLOOD	93.8	96.8	87.2	98.3	94.03	4.92	2.46

PERCENT RECOVERY OF TET IN DOG TISSUE
AT SIX CONCENTRATIONS

	0.1 mg/ml	0.5 mg/ml	1 mg/ml	5 mg/ml	10 mg/ml	20 mg/ml	MEAN	SD	SE
LIVER	90.7	90.2	88.8	101.5	96.0	93.2	93.4	4.7	1.9
KIDNEY	95.2	90.7	95.2	97.3	93.2	94.5	94.4	2.2	0.9
FAT	92.5	92.2	90.8	97.5	93.3	94.2	93.4	2.3	0.9
HEART	94.7	92.3	94.7	101.5	94.3	96.5	95.7	3.2	1.3
LUNG	93.7	90.2	92.7	98.0	98.2	94.8	94.6	3.1	1.3
MUSCLE		89.7	85.3	96.8	97.3	95.2	92.9	5.2	2.3
BRAIN		92.0	89.7	96.8	92.8	90.7	92.4	2.7	1.2
BLOOD	92.3	92.7	96.8	98.7	97.2	93.7	95.2	2.7	1.1

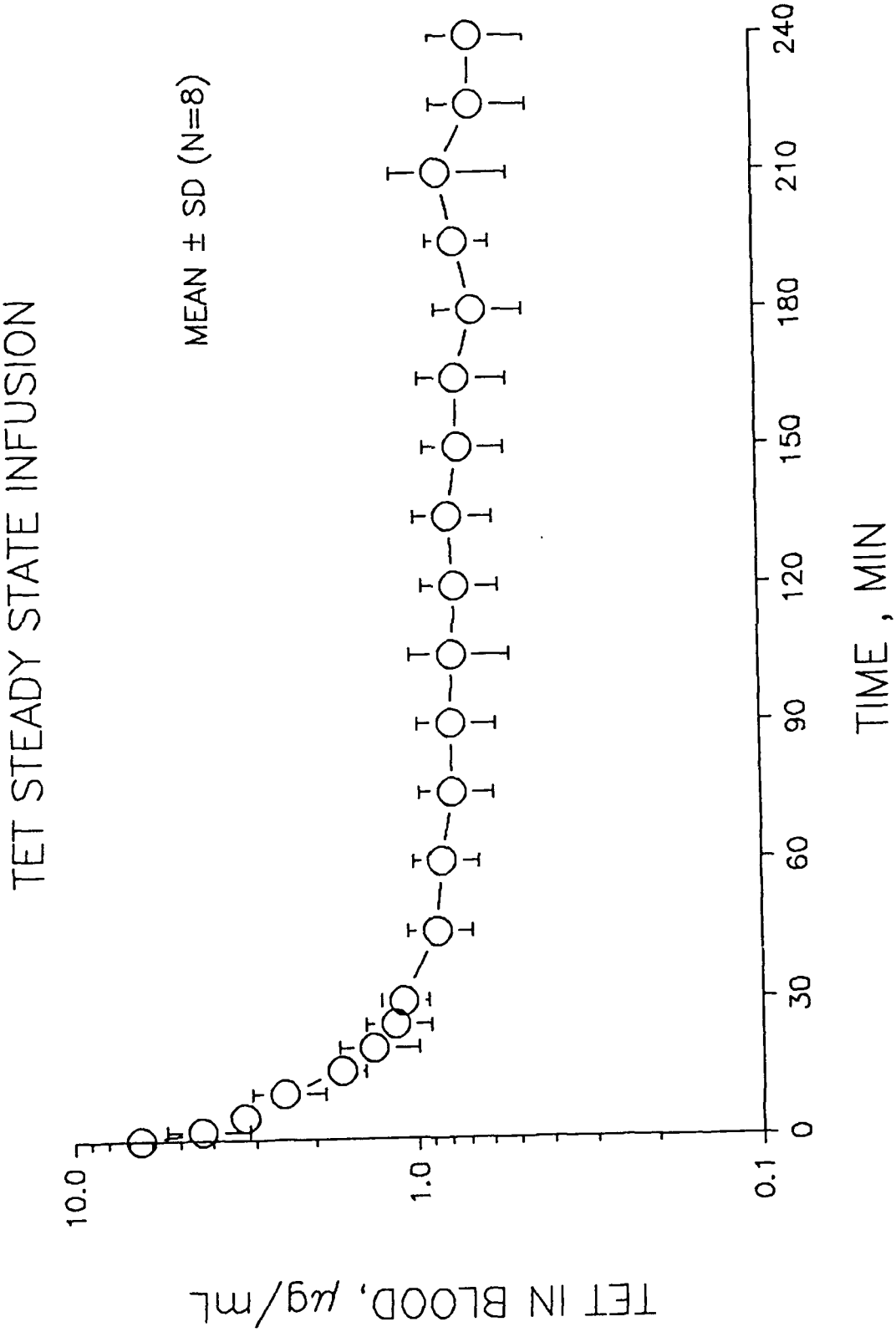
TABLE B-2

CONCENTRATIONS OF TET IN VARIOUS TISSUES OF DOGS

	UG/G#1	UG/G#2	UG/G#3	UG/G#4	UG/G#5	UG/G#6	UG/G#7	UG/G#8	MEAN	SD	SE
LIVER	3.200	0.502	0.901	3.168	1.848	4.117	4.952	4.816	2.938	1.706	0.603
KIDNEY	3.614	0.606	0.713	14.130	7.874	9.192	2.456	8.220	5.851	4.779	1.690
FAT	32.499	29.506	23.607	41.743	31.582	25.633	23.646	39.423	30.955	6.853	2.423
HEART	3.353	0.408	0.581	7.029	4.088	5.828	2.043	7.441	3.846	2.752	0.973
LUNG	3.013	0.310	0.636		4.742	6.434	1.371	3.310	2.477	2.306	0.815
MUSCLE	1.618	1.297	0.610	1.959	2.056	1.223	1.428	2.454	1.581	0.574	0.203
BRAIN	3.632	0.633	1.065	3.058	1.939	5.176	3.028	6.391	3.115	1.964	0.694
BLOOD ia	0.742	0.424	0.498	1.375	0.962	0.650	1.304	0.690	0.831	0.353	0.125

TABLE C-1

Fig. C-1


TET STEADY STATE INFUSION
MEAN ± SD (N=8)
TET IN BLOOD, μg/mL
10.0
1.0
0.1
0
30
60
90
120
150
180
210
240
TIME, MIN

PHARMACOKINETIC PARAMETERS OF PCE (160 mg/kg)
ADMINISTERED ORALLY TO RATS

	AUC ug•min/ml	T½ min.	hrs.	C_{max} ug/ml	T_{max} min.	CL ml.min/kg	V_{dapp} L/kg
RAT#1	10951	341.00	5.68	30.11	30.00	14.611	7.189
RAT#2	7645	601.34	10.02	23.16	45.00	20.930	18.161
RAT#3	9685	625.78	10.43	29.09	20.00	16.520	14.918
RAT#4	10231	514.58	8.58	17.18	15.00	15.639	11.612
RAT#5	11475	540.06	9.00	26.06	150.00	13.943	10.866
RAT#6	7059	401.43	6.69	23.63	10.00	22.666	15.130
MEAN	9508	504.03	8.40	24.71	45.00	17.385	12.646
SD	1787	112.08	1.87	4.53	52.92	3.572	3.737
SE	730	45.76	0.76	1.85	21.60	1.458	1.526

TABLE D-1

PHARMACOKINETIC PARAMETERS OF PCE (480 mg/kg)
ADMINISTERED ORALLY TO RATS

	TIME	AUC ug•min/ml	T½ min.	hrs.	C_{max} ug/ml	T_{max} min.	CL ml.min/kg	V_d L/kg
RAT#1	hrs. 36	27927	351.00	5.85	73.31	150.00	17.188	8.705
RAT#2	hrs. 96	24035	417.00	6.95	114.42	60.00	19.971	12.017
RAT#3	hrs. 36	22644	636.00	10.60	87.33	90.00	21.198	19.454
RAT#4	hrs. 36	22161	810.00	13.50	91.12	90.00	21.660	25.317
RAT#5	hrs. 72	25599	717.00	11.95	71.41	180.00	18.751	19.400
RAT#6	hrs. 96	29172	399.00	6.65	97.45	60.00	16.454	9.474
MEAN		25256	555.00	9.25	89.17	105.00	19.203	15.728
SD		2845	191.22	3.19	16.00	49.30	2.118	6.656
SE		1162	78.07	1.30	6.53	20.12	0.865	2.717

TABLE D-2

Fig. D-2

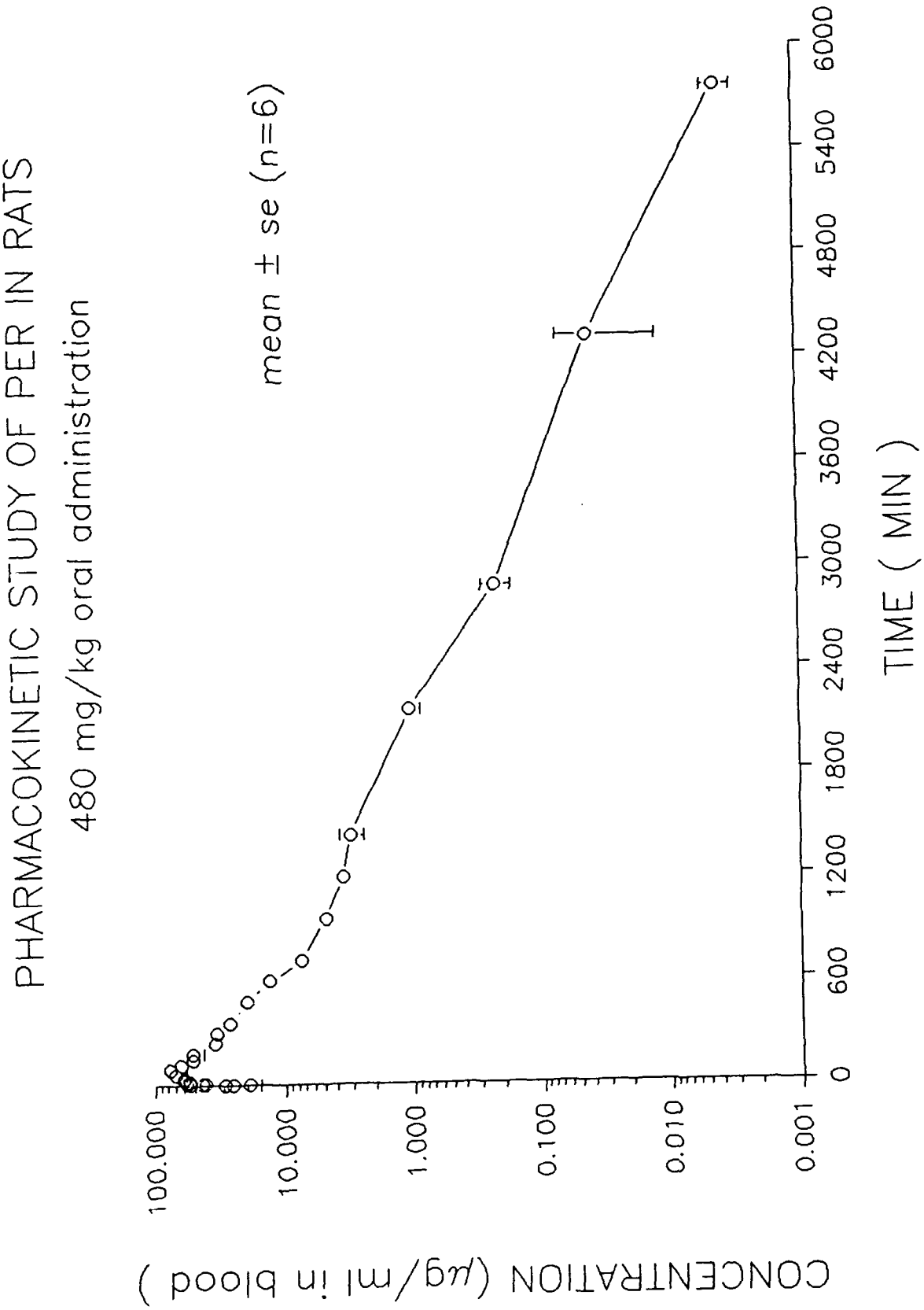

PHARMACOKINETIC STUDY OF PER IN RATS
480 mg/kg oral administration
mean ± se (n=6)
CONCENTRATION (μg/ml in blood)
100.000
10.000
1.000
0.100
0.010
0.001
0
600
1200
1800
2400
3000
3600
4200
4800
5400
6000
TIME (MIN)

Fig. D-3

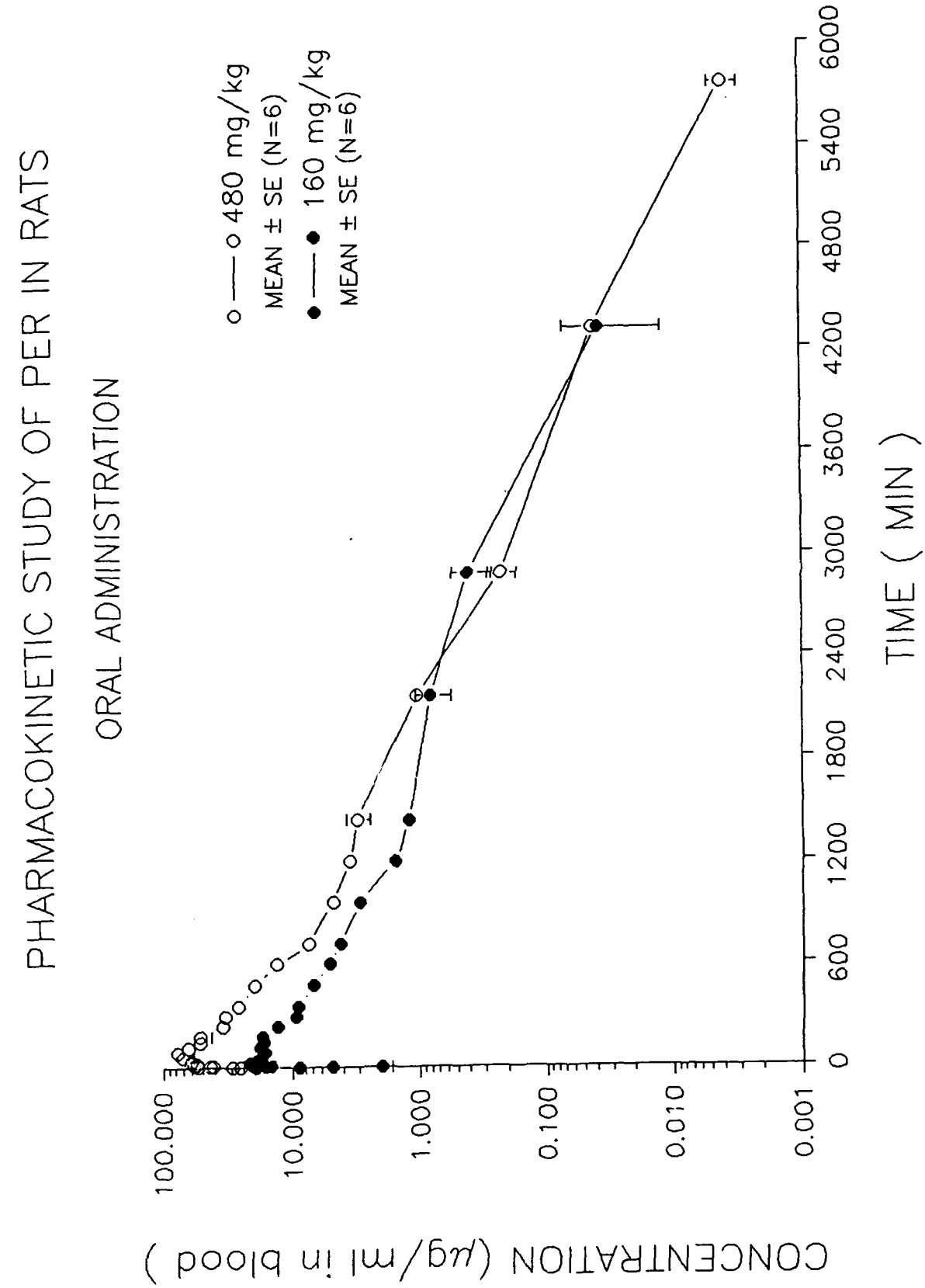

PHARMACOKINETIC STUDY OF PER IN RATS
ORAL ADMINISTRATION
o—— o 480 mg/kg
MEAN ± SE (N=6)
●—— ● 160 mg/kg
MEAN ± SE (N=6)
CONCENTRATION (μg/ml in blood)
100.000
10.000
1.000
0.100
0.010
0.001
0
600
1200
1800
2400
3000
3600
4200
4800
5400
6000
TIME (MIN)

Fig. D-4

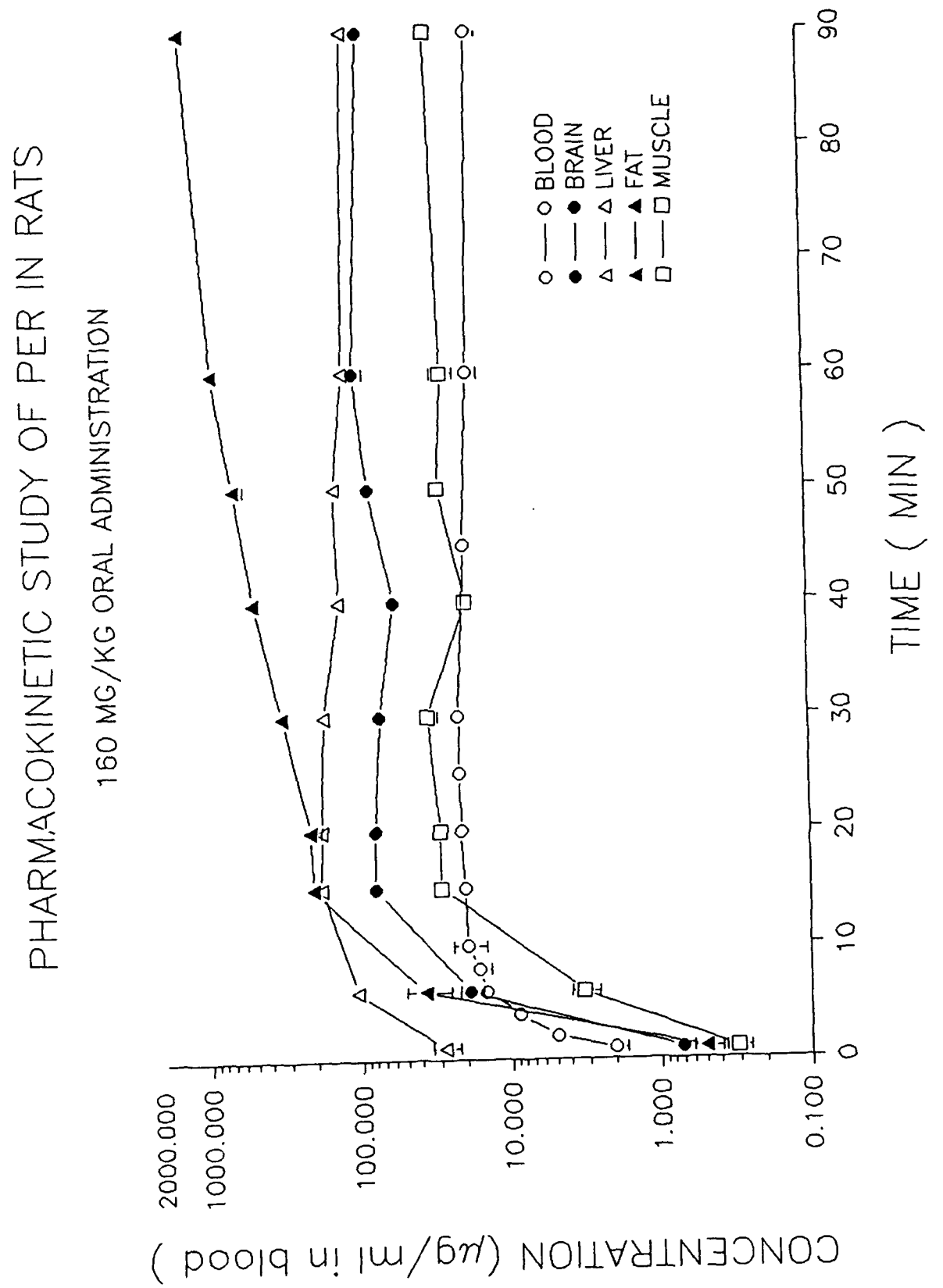

PHARMACOKINETIC STUDY OF PER IN RATS
160 MG/KG ORAL ADMINISTRATION
BLOOD
BRAIN
LIVER
FAT
MUSCLE
2000.000
1000.000
100.000
10.000
1.000
0.100
CONCENTRATION (μg/ml in blood)
0
10
20
30
40
50
60
70
80
90
TIME (MIN)

Fig. D-5

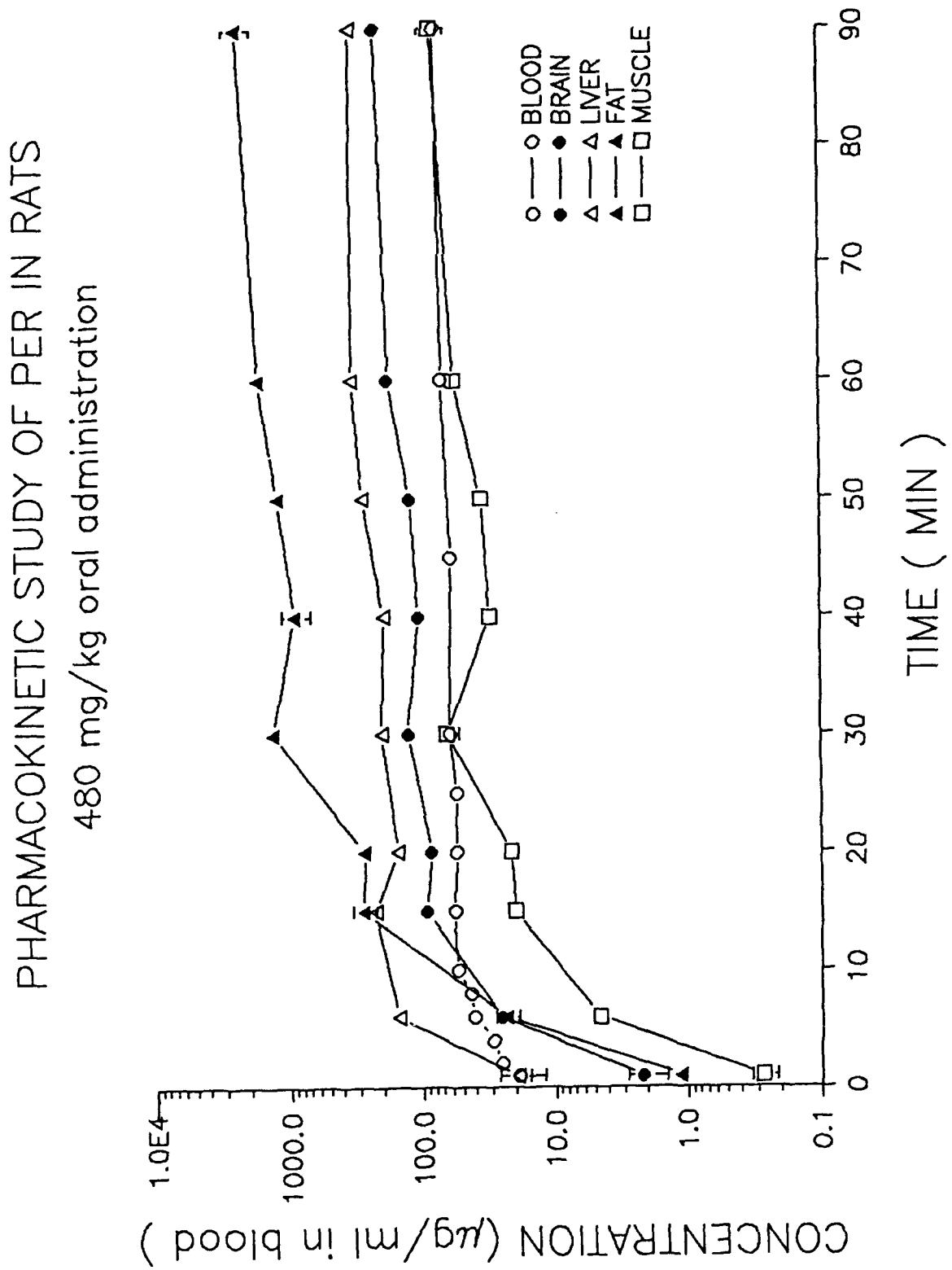

PHARMACOKINETIC STUDY OF PER IN RATS
480 mg/kg oral administration
CONCENTRATION (μg/ml in blood)
TIME (MIN)
BLOOD
BRAIN
LIVER
FAT
MUSCLE
1.0E4
1000.0
100.0
10.0
1.0
0.1
0
10
20
30
40
50
60
70
80
90

Fig. D-6

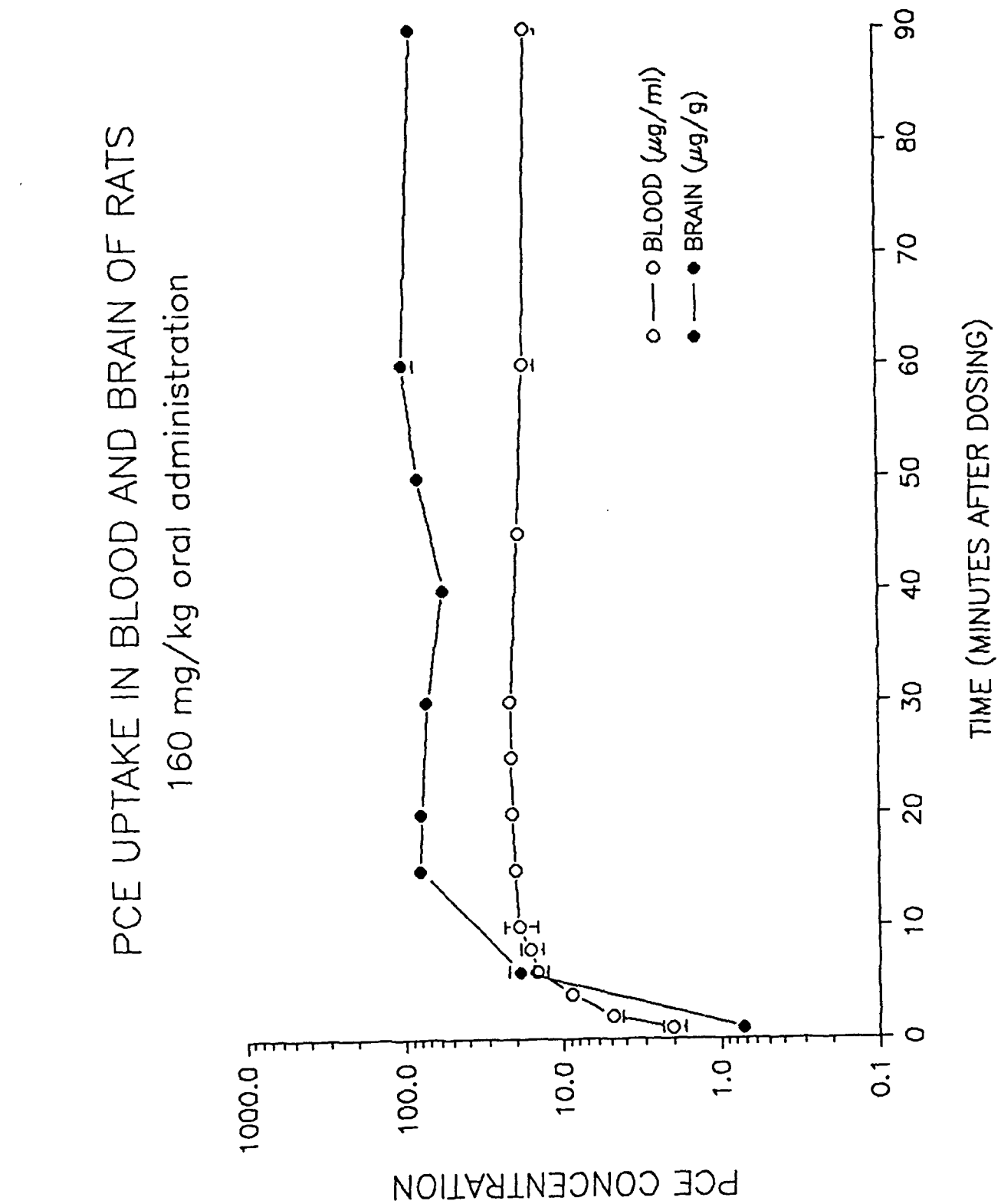
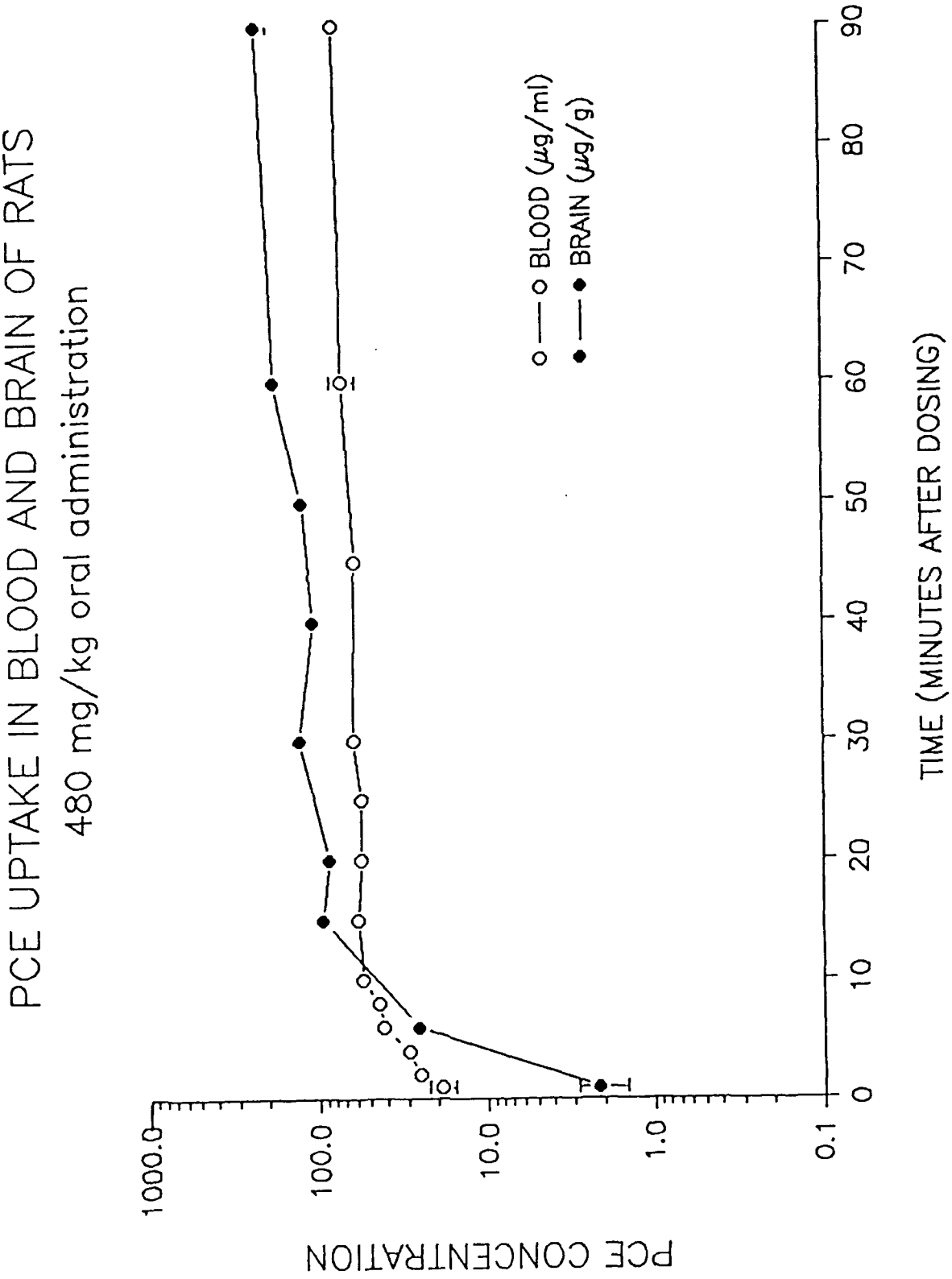

PCE UPTAKE IN BLOOD AND BRAIN OF RATS
160 mg/kg oral administration
PCE CONCENTRATION
1000.0
100.0
10.0
1.0
0.1
0
10
20
30
40
50
60
70
80
90
TIME (MINUTES AFTER DOSING)
BLOOD (μg/ml)
BRAIN (μg/g)

Fig. D-7


PCE UPTAKE IN BLOOD AND BRAIN OF RATS
480 mg/kg oral administration
BLOOD (μg/ml)
BRAIN (μg/g)
PCE CONCENTRATION
1000.0
100.0
10.0
1.0
0.1
0
10
20
30
40
50
60
70
80
90
TIME (MINUTES AFTER DOSING)

PARAMETERS FOR PBPK MODEL SIMULATIONS OF PCE TISSUE PHARMACOKINETICS IN THE RAT

The physiologically-based pharmacokinetic model for PCE is taken from [1] with two major differences:

a. the elimination from the liver is not linear but Michealis-Menten type,
b. the richly perfused compartment is divided into three compartments: heart, kidney, and brain, and a lung tissue compartment was added.

The subscripts in the model and in the other part of the text are as follows:

H	-	heart	BR	-	brain
K	-	kidney	M	-	muscle
R	-	rest of body	Li	-	liver
F	-	fat	L	-	lung
A	-	alveolar space	V	-	venous blood
BL	-	arterial blood	T	-	total (blood)

Constants of the model for 339 g rat

Tissue volumes (ml)			Blood flows (ml/min)		
V_{BL}	=	25.1	Q_T	=	115.5
V_{BR}	=	2.0	Q_{BR}	=	2.6
V_M	=	119.1	Q_M	=	32.2
V_{Li}	=	11.5	Q_{Li}	=	17.9
V_H	=	1.1	Q_H	=	6.0
V_K	=	2.6	Q_K	=	16.2
V_R	=	150.9	Q_R	=	32.6 (different from [1] to satisfy Q_T)
V_F	=	24.4	Q_F	=	8.0

Fig. D-8

Physiological parameters from reference values

alveolar space volume,	V_a	=	1.9 ml (Dallas et al., 1991)
alveolar ventilation rate,	VR_a	=	169.4 ml/min (Dallas et al., 1991)
lung:alveolar mass transfer coefficient,	h	=	500 ml/min (Angelo and Pitchard 1984)
Michaelis-Menten constants	k_m	=	2.9378 ug/min (Gargas et al., 1986)
	V_m	=	5.86 ug/ml (Gargas et al., 1986)

Parameters calculated from observations

Dose = 3350 µg
Average rat size = 339 g

Calculations of tissue partition coefficients:

lung:air	46.87
fat	152.54
lung	2.48
liver	4.96
muscle	2.98
brain	4.24
heart	2.68
kidney	4.45
rest of body	2.98

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL OF PCE INTRAARTERIAL ADMINISTRATION

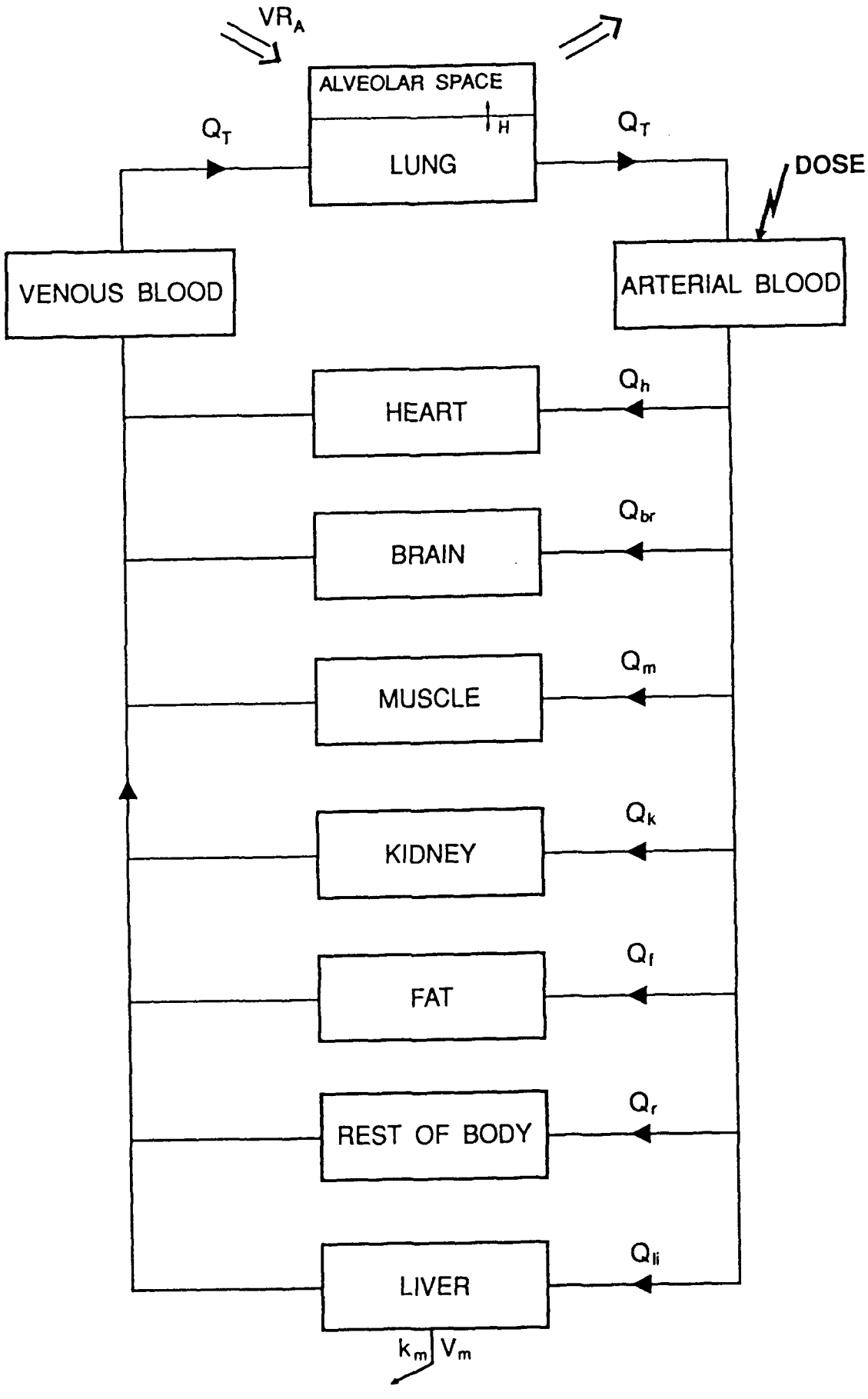


Fig. D-10

Differential equation system

Non-eliminating organs:

$$V_i \frac{dC_i}{dt} = Q_i \left(C_T - \frac{C_i}{R_i} \right)$$

where i= H; BR; K; M; R; F

arterial blood

$$V_{BL} \frac{dC_{BL}}{dt} = Q_T \left(\frac{C_L}{R_L} - C_{BL} \right)$$

lung

$$V_L \frac{dC_L}{dt} = Q_T \left(C_V - \frac{C_L}{R_L} \right) + h \left(C_A - \frac{C_L}{R_A} \right)$$

liver

$$V_{Li} \frac{dC_{Li}}{dt} = Q_{Li} \left(C_{BL} - \frac{C_{Li}}{R_{Li}} \right) - \frac{V_m \frac{C_{Li}}{R_{Li}}}{k_m + \frac{C_{Li}}{R_{Li}}}$$

alveloar space

$$V_A \frac{dC_A}{dt} = h \left(\frac{C_L}{R_A} - C_A \right) - VR_A C_A$$

venous blood

$$V_{BL} \frac{dC_V}{dt} = \sum Q_i \frac{C_i}{R_i} - Q_T C_V$$

where i=H; BR; Li; K; M; F; R

Fig. D-11

Calculation of partition coefficients from intraarterial data

R_R and R_A are taken from [1]

The part. coeff. of noneliminating compartments (except the lung) are calculated according to [2]:

$$R_i = \frac{AUC_i}{AUC_{BL}}$$

where i=H; BR; K; R; F

R_L is calculated integrating the differential equation of arterial blood:

$$\int_0^\infty V_{BL}\frac{dC_{BL}}{dt}dt = Q_T\left(\frac{\int_0^\infty C_L dt}{R_L} - \int_0^\infty C_{BL}dt\right)$$

$$V_{BL}C_{BL}^0 = Dose = Q_T\left(\frac{AUC_L}{R_L} - AUC_{BL}\right)$$

$$R_L = \frac{AUC_L}{\frac{Dose}{Q_T} + AUC_{BL}}$$

REFERENCES

[1] Dallas, C.E., Gallo, J.M., Ramanathan, R., Muralidhara, S., and Bruckner, J.V. Physiological pharmacokinetic modeling of inhaled trichloroethylene in rats. Toxicol. Appl. Pharmacol. 110, 303-314 (1991).

[2] Gallo, J.M., Lam, F.C., and Perrier, D.G. Area method for the estimation of partition coefficients for physiological pharmacokinetic models. J. Pharmacokin. Biopharm. 15, 271-280 (1987).

[3] Gargas, M.L., Andersen, M.E., and Clewell, H.J., III. A physiologically based simulation approach for determining metabolic constants from gas uptake data. Toxicol. Appl. Pharmacol. 86, 341-352 (1986a).

[4] Angelo, M.J. and Pritchard, A.B. Simulations of methylene chloride pharmacokinetics using a physiologically based model. Reg. Toxicol. Pharmacol. 4, 329-339 (1984).

PHARMACOKINETIC PARAMETERS

PCE INTRAARTERIAL ADMINISTRATION (10 MG/KG)

	T½ hrs	C_{max} (ug/g)	T_{max} (min)	Observed AUC ug·min/g	Predicted AUC ug·min/g
LIVER	6.5	40.96	1	1736	1899
KIDNEY	6.9	36.02	1	1758	1851
FAT	7.8	64.42	30	60334	66635
HEART	7.3	18.59	1	1058	1115
LUNG	8.0	11.34	1	908	960
MUSCLE	7.4	5.66	10	1177	1239
BRAIN	7.4	21.66	1	1729	1763
BLOOD	8.3	4.63	1	395	416

Fig. D-13

Fig. D-14

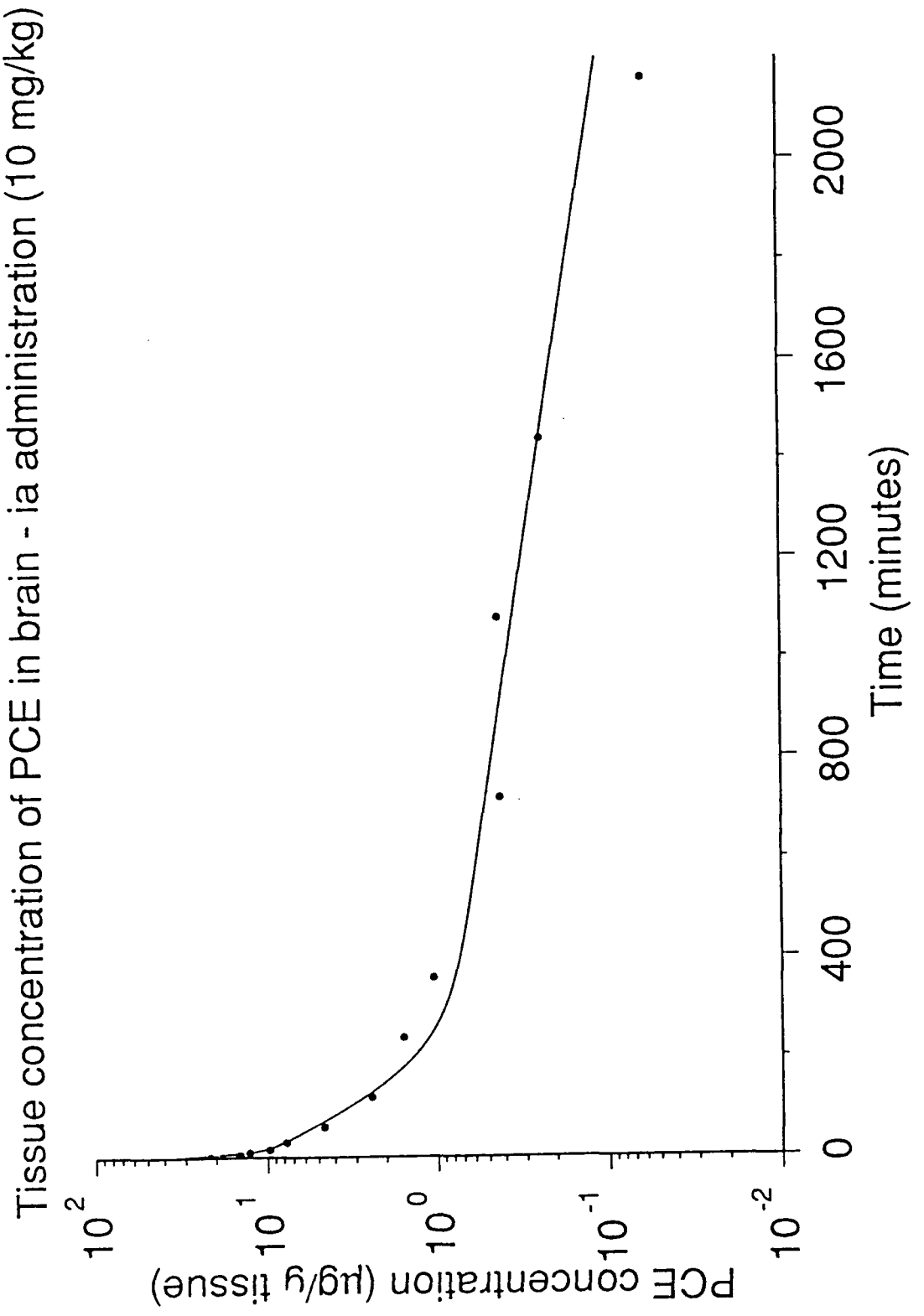

Tissue concentration of PCE in brain - ia administration (10 mg/kg)
PCE concentration (µg/g tissue)
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
0
400
800
1200
1600
2000
Time (minutes)

Fig. D-15

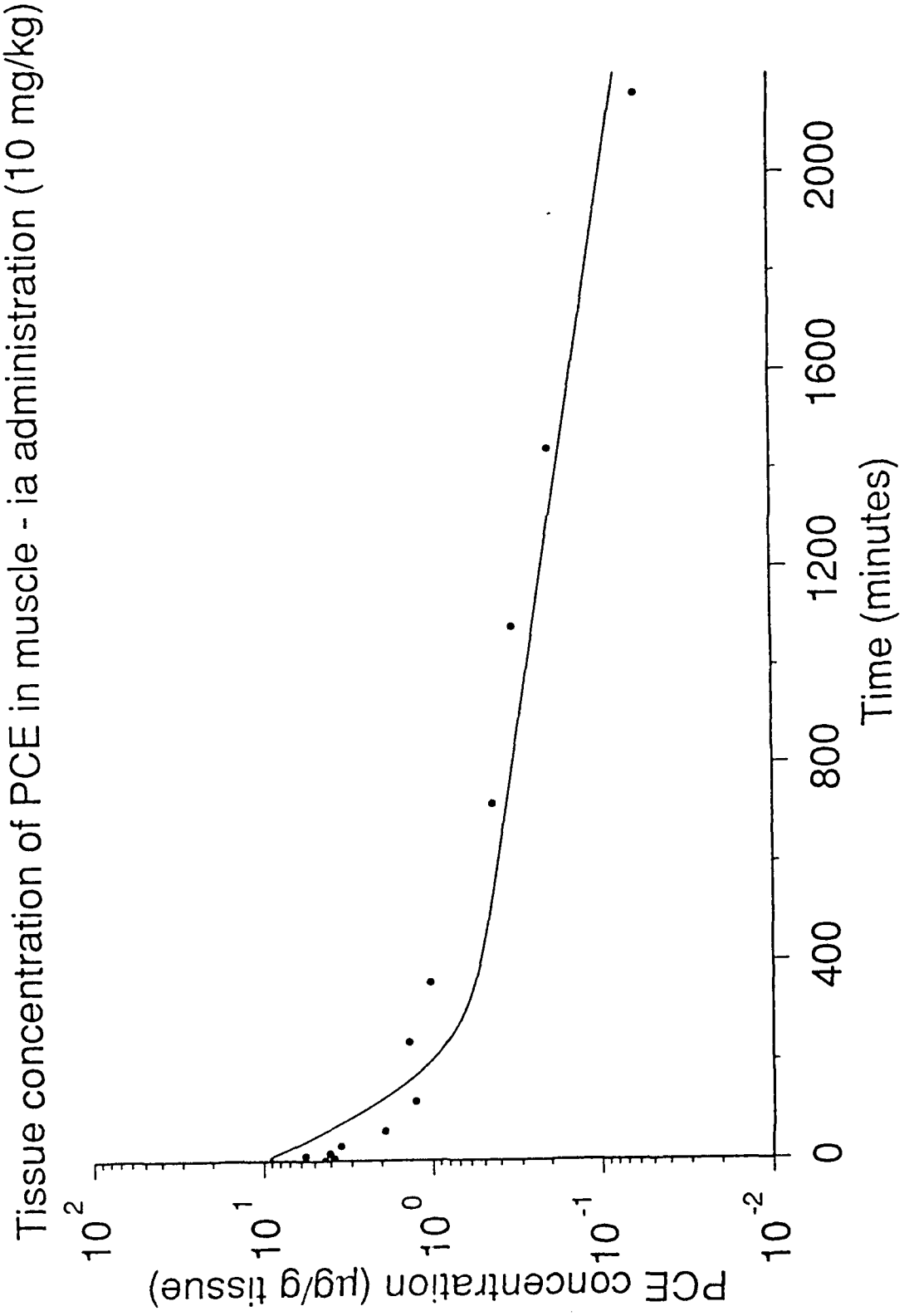

Tissue concentration of PCE in muscle - ia administration (10 mg/kg)
PCE concentration (µg/g tissue)
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
0
400
800
1200
1600
2000
Time (minutes)

Fig. D-16

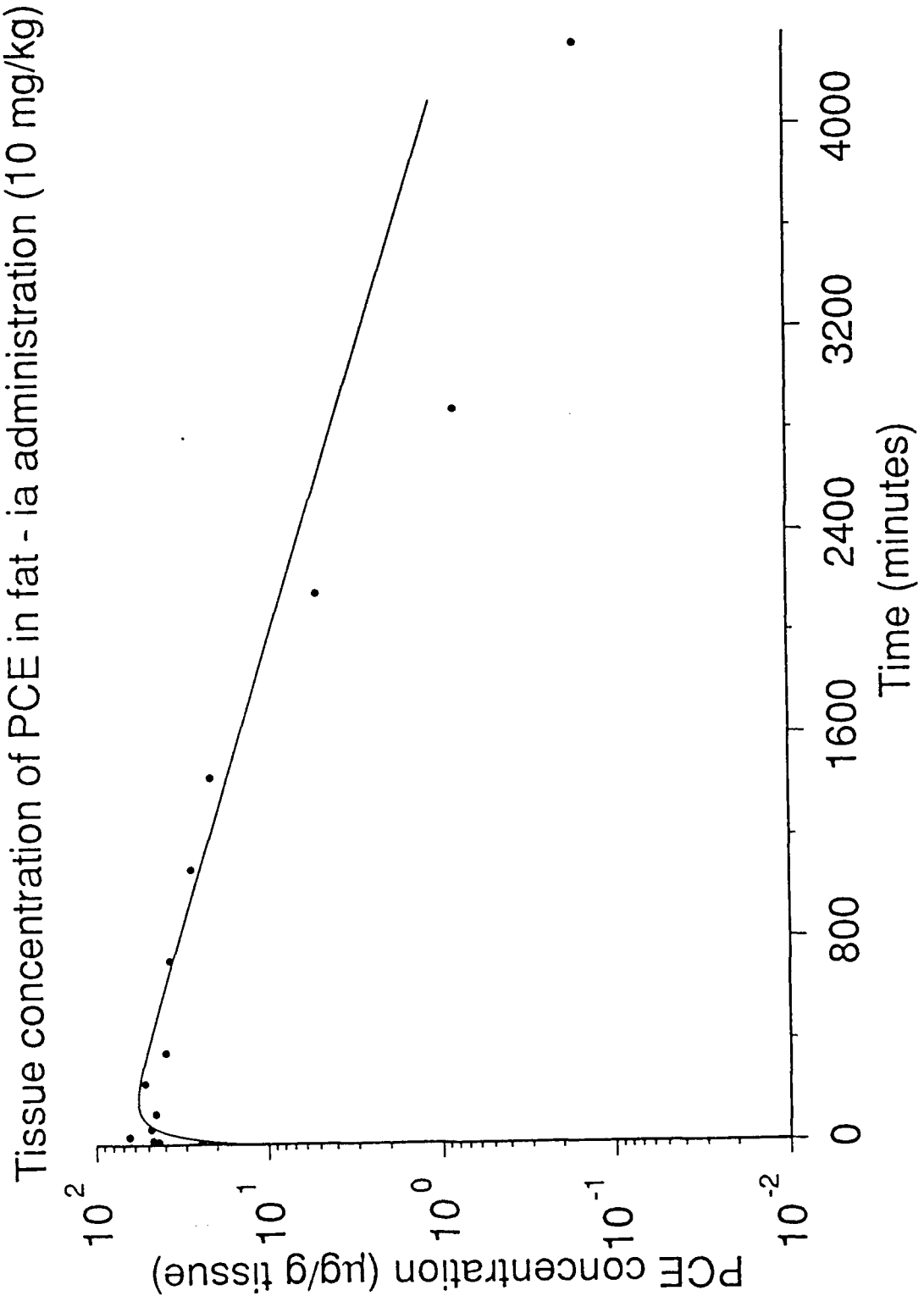

Tissue concentration of PCE in fat - ia administration (10 mg/kg)
PCE concentration (µg/g tissue)
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
0
800
1600
2400
3200
4000
Time (minutes)

Fig. D-17

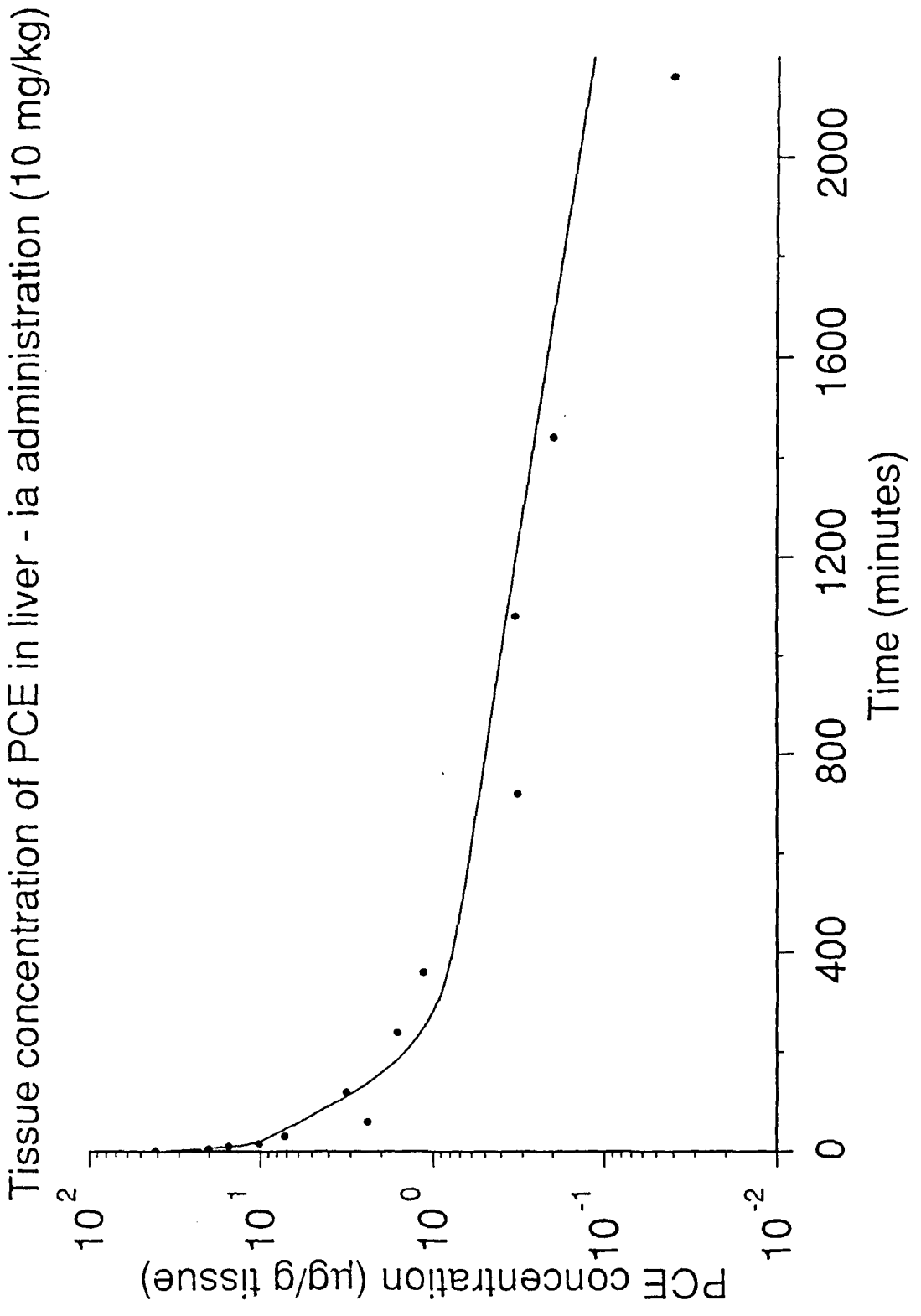

Tissue concentration of PCE in liver - ia administration (10 mg/kg)
PCE concentration (µg/g tissue)
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
0
400
800
1200
1600
2000
Time (minutes)

Fig. D-18

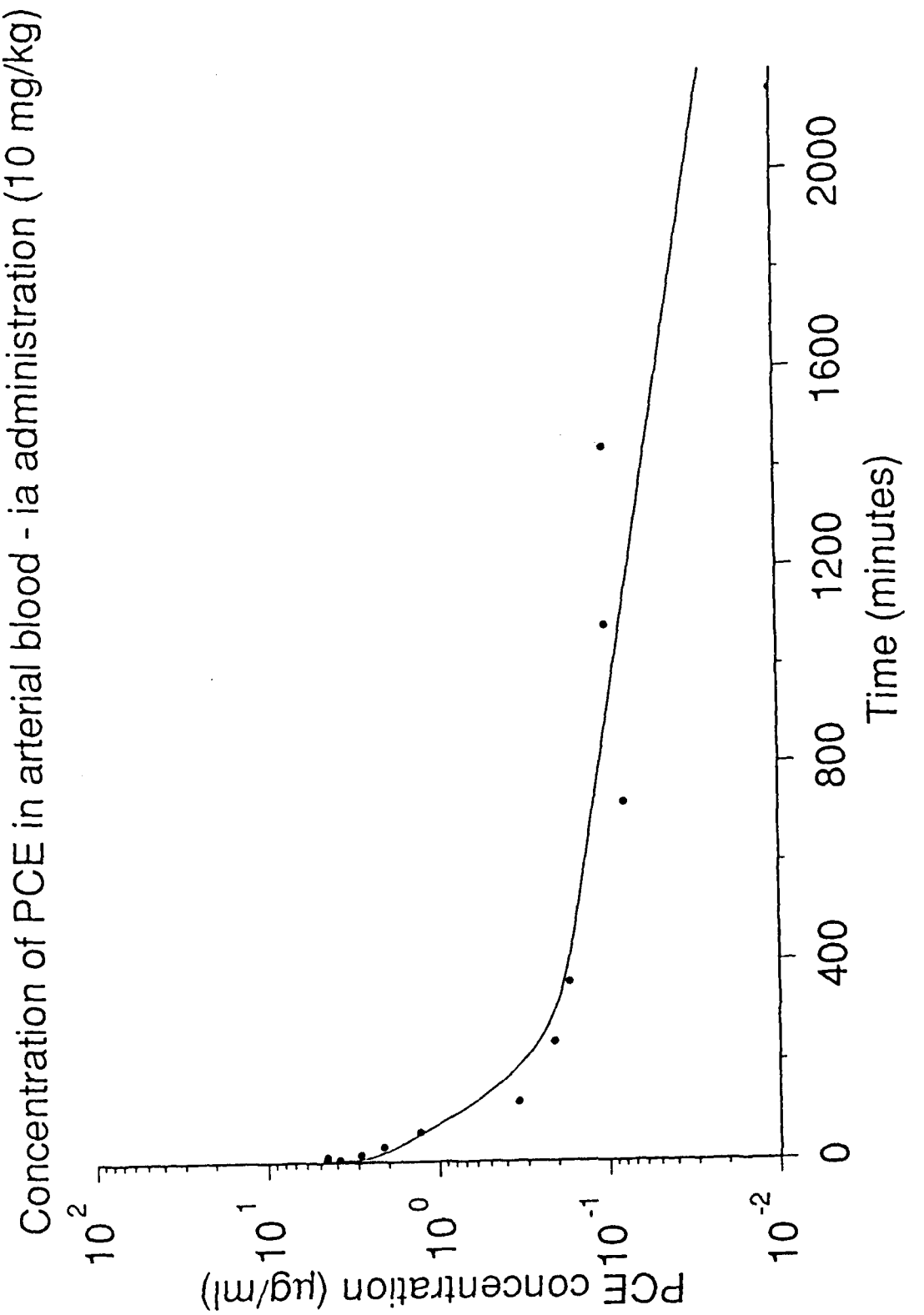

Concentration of PCE in arterial blood - ia administration (10 mg/kg)
PCE concentration (µg/ml)
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
0
400
800
1200
1600
2000
Time (minutes)

Fig. D-19

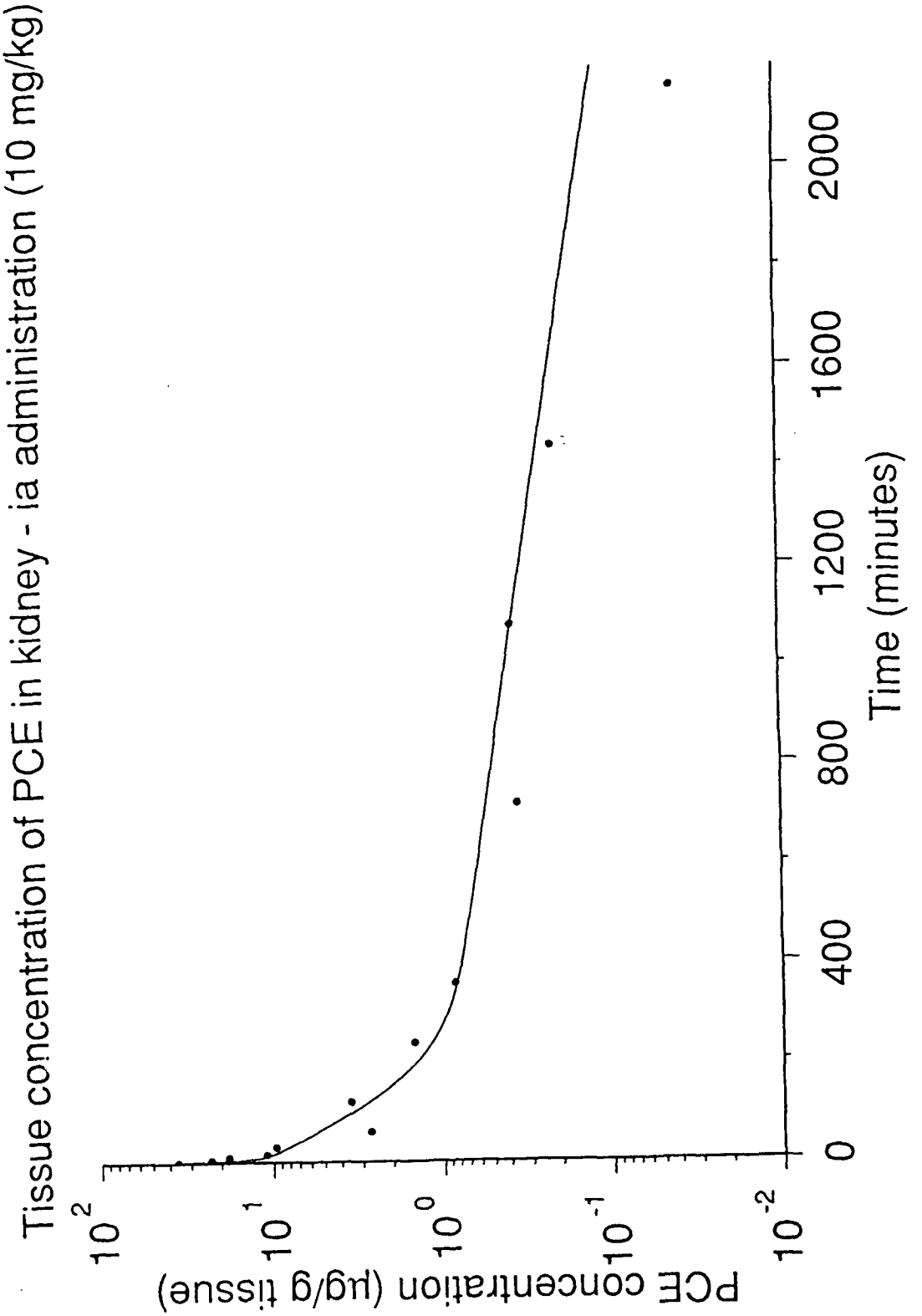

Tissue concentration of PCE in kidney - ia administration (10 mg/kg)
PCE concentration (µg/g tissue)
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
0
400
800
1200
1600
2000
Time (minutes)

Fig. D-20

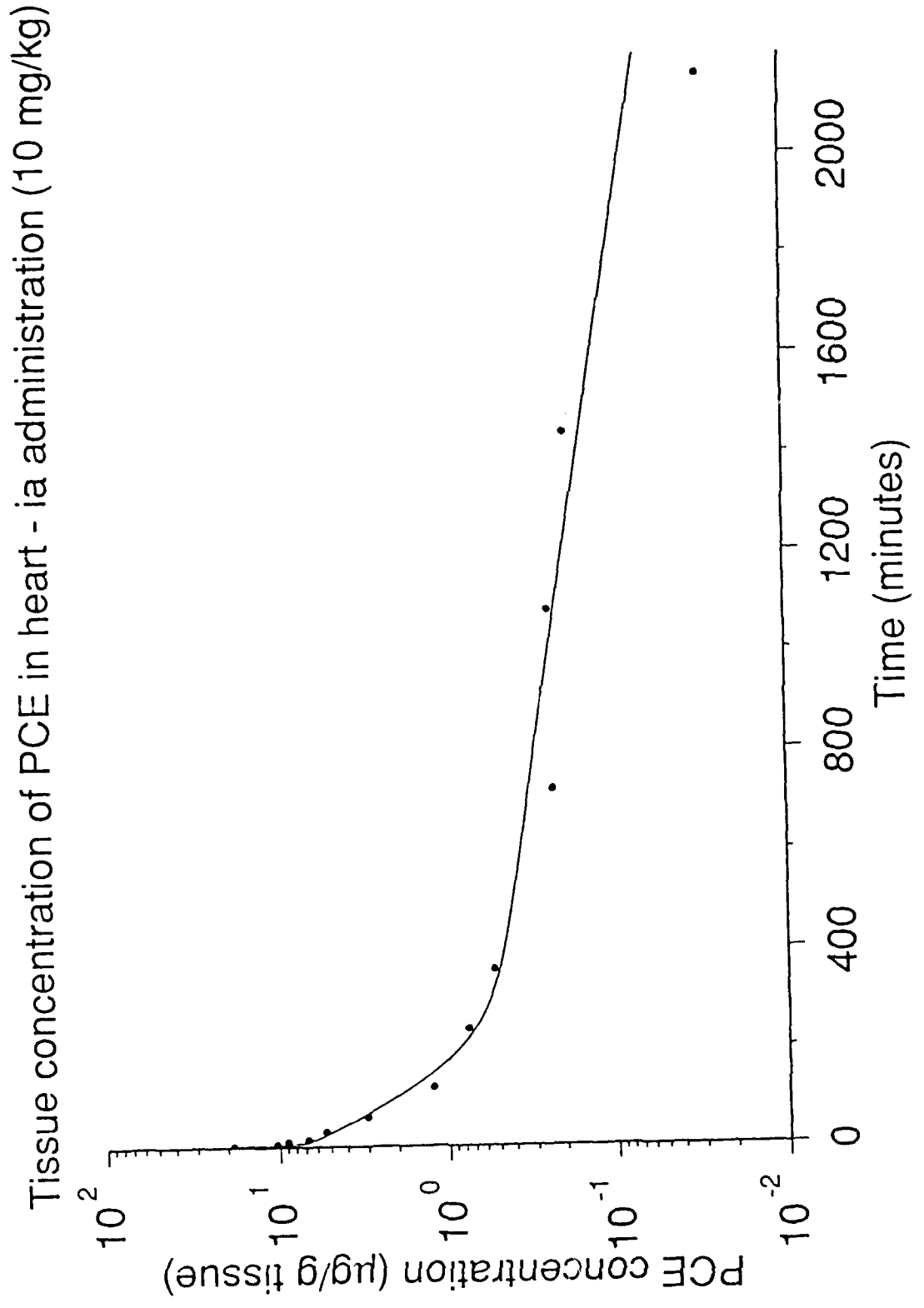
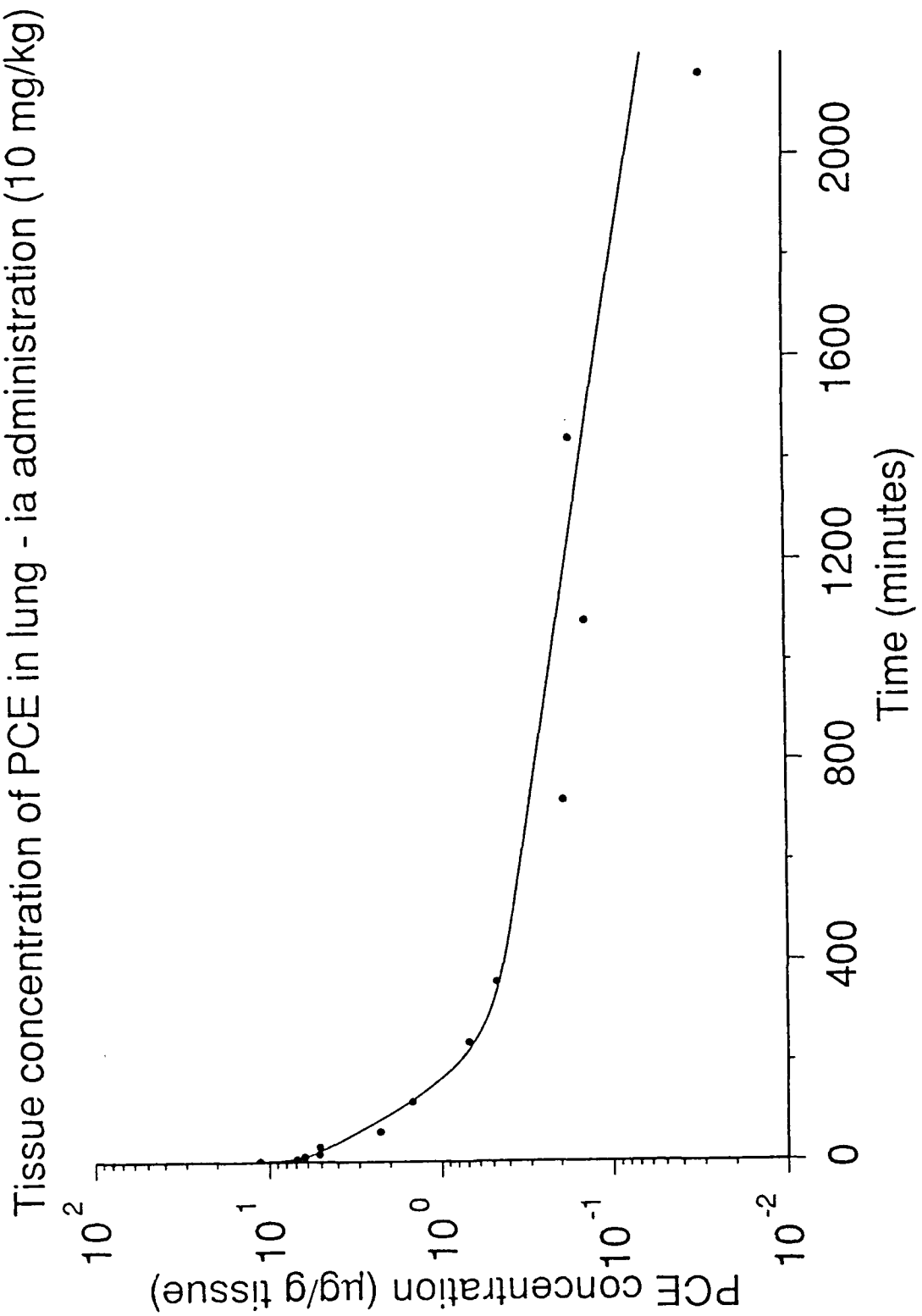

Tissue concentration of PCE in heart - ia administration (10 mg/kg)
PCE concentration (µg/g tissue)
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
0
400
800
1200
1600
2000
Time (minutes)

Fig. D-21


Tissue concentration of PCE in lung - ia administration (10 mg/kg)
PCE concentration (µg/g tissue)
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
0
400
800
1200
1600
2000
Time (minutes)

RESULTS AND CONCLUSIONS

1. Due to the highly lipophilic nature of PCE, the maximum tissue concentration (C_{max}) in the fat was substantially higher (ranging from 1.6 to 11.4 times greater) than the other tissues. The tissue with the lowest rate of blood perfusion of those sampled, muscle, had the lowest C_{max}. The relative importance of perfusion in the tissue pharmacokinetics of PCE provided additional verification to the perfusion-limited approach to a PBPK model for PCE.

2. The area under the tissue concentration-time curve (AUC) for fat tissues was between 34 and 67 times greater than the AUC for liver, kidney, heart, lung, muscle, and brain. The tissue AUCs for liver, kidney, and brain were very similar, reflecting their similarity in a high rate of blood perfusion.

3. The predictions of tissue AUCs for PCE were generally within 5% of the AUCs calculated from the observed tissue concentrations over time, except for liver and fat which had predicted tissue AUCs within approximately 9% of the observed tissue AUC.

4. In comparisons with observed tissue concentration-time data, tissue concentrations of PCE were well predicted by the PBPK model in brain, blood, kidney, heart, and lung over the length of the time course following PCE administration. In the first minutes following PCE exposure fat tissue levels were underpredicted, followed by fairly accurate predictions up to 84 hours, and terminal time points that were overpredicted relative to the observed fat PCE concentration. There was an initial over prediction of PCE concentrations in the muscle tissues up to 3 hours following administration, with more accurate predictions thereafter.

5. The PBPK model for PCE was therefore demonstrated to have considerable utility in accurately predicting tissue levels in rats. The least accurate predictions occurred in the most poorly perfused tissue sampled, muscle, and in the fat tissues more than 3 days following exposure. Tissue AUC proved to be a parameter accurately predicted by the PBPK model, which may be of significant utility in defining the applicability of this and similar models in risk assessments pertaining to specific compartments of the model.

ABSTRACT

Perchloroethylene (PCE) given prior to operant testing was assessed for its ability to produce tissue dose-response relationships for use in the development of a toxicodynamic model. Food-restricted, male Sprague-Dawley rats were shaped to lever press for an evaporated milk presentation. After stable baseline response rates were established, rats were given a 1 ml oral bolus of PCE in a 10% aqueous Emulphor® emulsion. Polyethylene glycol (PEG) 400 and corn oil were evaluated to a lesser extent as dosing vehicles. Immediately after dosing, rats were placed in a computer-controlled and monitored operant test cage for 90 minutes. The rate of post-exposure responding was then compared to the baseline response rate. Additionally, pharmacokinetic profiles of PCE were determined in blood and tissues. PCE (80-640 mg/kg) decreased the rate of fixed-ratio (FR) responding almost immediately, but rats quickly resumed a normal response rate despite increasing blood and brain concentrations of the depressant chemical. Similar recovery times were seen for all doses. The finding that normal behavior returned in the presence of increasing brain concentrations suggested that the development of acute tolerance was responsible for the rapid recovery. Thus, a discernable relationship between the brain concentration of PCE and the observed neurobehavioral deficit was not detected by the FR-40 schedule. The onset of behavioral depression was, however, dependent on the dosing vehicle. Behavioral depression occurred more rapidly after administration of PCE in an aqueous Emulphor® emulsion than in PEG 400 or corn oil, both of which delayed the absorption of PCE. The amount of operant training rats received influenced their response to PCE. The depressant action of PCE on FR responding was either fully or partially blocked by increasing the amount of training. This finding supported the contention that the behavioral effects observed were centrally mediated and not due to a peripheral action of PCE. (Supported by AFOSR 910356 and 1991 NDSEG Fellowship).

Fig. E-1

INTRODUCTION

Exposures to volatile organic compounds (VOCs) result from their widespread commercial use and improper disposal. VOC exposures may produce central nervous system (CNS) depressant effects such as dizziness, confusion, short-term memory deficits, unconsciousness, and death. The intensity and time course of these CNS effects are assumed to be dependent upon the level of VOC received by the brain. Due to the lack of brain concentration data for most VOCs, including PCE, relationships between brain dose and response must be inferred from animal studies. To impart a more scientific basis to such extrapolations, physiologically-based pharmacokinetic (PB-PK) models are being developed that describe a chemical's dynamics in the blood and specific organs. Furthermore, the chemical's effects are being quantified and used to establish biological response or toxicodynamic models. Such toxicodynamic models would allow, for example, the prediction of neurobehavioral toxicity by inputting brain VOC concentrations that were generated from PB-PK models. Once an accurate PB-PK model exists and the relationship between brain concentration and neurobehavioral toxicity is established, a toxicodynamic model can be used to predict human neurobehavioral effects for a given exposure. Such predictions could then form the basis for a rational approach to setting exposure limits.

The investigation of neurobehavioral alterations is desirable for two reasons: 1) such effects are frequently used by regulatory agencies as the basis for deriving exposure standards for VOCs and 2) CNS depression can be correlated with the concentration of parent compound in the brain. PCE was selected for use because it is poorly metabolized and has a relatively long biological half-life relative to other halocarbons. Additionally, substantial human dose-response data that may be used to further validate a toxicodynamic model for use in human risk assessment exist.

OBJECTIVES

1. To determine the influence of dosing vehicle and extent of operant training on PCE-induced neurobehavioral effects.

2. To determine the effect of several doses of PCE on the time course and magnitude of the neurobehavioral response.

3. To characterize the relationship between blood and brain concentrations of PCE and altered behavior.

4. To assess the utility of oral bolus dosing and operant testing on a FR schedule to delineate tissue dose-response relationships for use in the development of a toxicodynamic model.

Fig. E-3

BEHAVIORAL MONITORING METHOD

Shaping and Baseline Response Rates

Male Sprague-Dawley rats (300-350 g) were food-restricted (10 g/day) during a period in which they were shaped to lever-press for evaporated milk presentation (0.05 ml) on a FR-40 schedule. Rats were shaped daily in sessions that ranged from 30-90 minutes. Baseline responding rates were determined during 90-minute operant sessions spaced 24 hours apart. The first baseline rate was determined following no treatment and the second after receipt of a 1 ml oral bolus of dosing vehicle (10% aqueous Emulphor® emulsion) which served as a control.

Neurobehavioral Monitoring Following PCE Exposure

Twenty-four hours after completion of the second baseline measurement, rats were given a 1 ml oral bolus of dosing vehicle with PCE (50-1600 mg/kg), and immediately placed in an operant test cage for 90 minutes. The number of responses in each 5-minute segment of the operant session was computer-recorded. Since it was established that dosing vehicle alone had no effect on responding, response rates of PCE-treated rats were calculated as a percentage of their individual baseline values.

Fig. E-4

DETERMINATION OF BLOOD AND TISSUE PHARMACOKINETICS

Blood

Male Sprague-Dawley rats (300-350 g) were food-restricted (10 g/day) for 72 hours prior to being surgically implanted with an indwelling carotid artery cannula. After an overnight recovery period, rats received a 1 ml oral bolus of either 160 or 480 mg/kg PCE in a 10% aqueous Emulphor® emulsion. Serial blood samples were collected via the carotid artery cannula at 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 45, 60, and 90 minutes following oral dosing.

Tissues

Male Sprague-Dawley rats (300-350 g) were food-restricted (10 g/day) for 72 hours prior to receiving a 1 ml oral bolus of either 160 mg/kg PCE in a 10% aqueous Emulphor® emulsion or 480 mg/kg PCE in a 20% aqueous Emulphor® emulsion. Animals were serially sacrificed by decapitation at 1, 6, 15, 20, 30, 40, 50, 60, and 90 minutes post dosing. Samples of brain, liver, fat and muscle were removed immediately and placed in chilled scintillation vials containing 2 ml of saline and 8 ml of isooctane. Tissues were homogenized, vortexed, and centrifuged.

Fig. E-5

Analytical Methods

Blood samples and aliquots of the tissue supernatant were diluted in saline and placed in 20 ml headspace vials. Vials were capped and subjected to controlled temperature and pressure conditions in the HS-101 Headspace Sampler unit of a Perkin Elmer Model 8500 gas chromatograph. Analysis of PCE in the vial headspace was conducted utilizing a stainless- steel column packed with 3% FFAP and an electron capture detector. Operating temperatures were 360°C, detector; 90°C, column; 90°C, headspace; and 200°C, injector. Concentrations were calculated from a standard curve and corrected for the percent recovery characteristic of blood and tissue samples.

RESULTS

1. Operant training on a FR-40 schedule produced animals with stable baseline response rates. Response rates were not influenced by oral administration of 1 ml of the dosing vehicle.

2. PCE-induced neurobehavioral effects were influenced by the dosing vehicle. The use of PEG 400 resulted in a slight delay in the onset of behavioral depression relative to the aqueous Emulphor® emulsion. Corn oil resulted in an even longer delay than did PEG 400 (Fig. 1).

3. PCE given as a single, oral bolus at doses of 80-640 mg/kg caused a transient depression of operant responding. A dose of 50 mg/kg was ineffective and 720 mg/kg produced gross ataxia (Fig. 2 and Fig. 3).

4. When PCE was given in an aqueous Emulphor® emulsion, response rates declined almost immediately but recovered to baseline levels within 40 minutes. The immediate decline in responding can be explained by detectable levels of PCE in the brain as early as 1 minute after dosing. The rapid recovery, however, did not coincide with a declining brain concentration, since PCE levels either increased or remained constant over the duration of the operant session (Fig. 4 and Fig. 5). Recovery time was remarkably similar for all doses, with most animals recovering within 20 minutes of one another despite an 8-fold difference in dose.

5. PCE-induced neurobehavioral effects were also influenced by the amount of previous training. The behavioral depressant action of both the 160 and 480 mg/kg doses was fully and partially blocked, respectively, by increasing the amount of training rats received prior to dosing (Fig. 6). This phenomenon was seen in rats that were trained on average for an additional 184 minutes over 7 days, including 4 days after the FR-40 criterion was reached.

SUMMARY

1. A single, oral bolus dose of PCE produced a neurobehavioral effect that was detectable with operant testing on a FR-40 schedule. The neurobehavioral effect was manifest as a transient decrease in response rate.

2. The dosing vehicle was a critical factor in determining the time course of neurobehavioral deficit.

3. An increase in the amount of operant training appeared to raise the threshold dose for PCE-induced neurobehavioral effect.

4. Blood and brain concentrations of PCE rapidly increased immediately after dosing and quickly achieved a near steady state equilibrium which persisted for the remainder of the operant session.

5. Administration of single, oral bolus doses of PCE (80-640 mg/kg) did not appear to result in a proportional relationship between the brain concentration of PCE and the neurobehavioral deficit.

VEHICLE EFFECT ON FIXED-RATIO RESPONDING FOLLOWING A SINGLE, ORAL BOLUS OF PCE

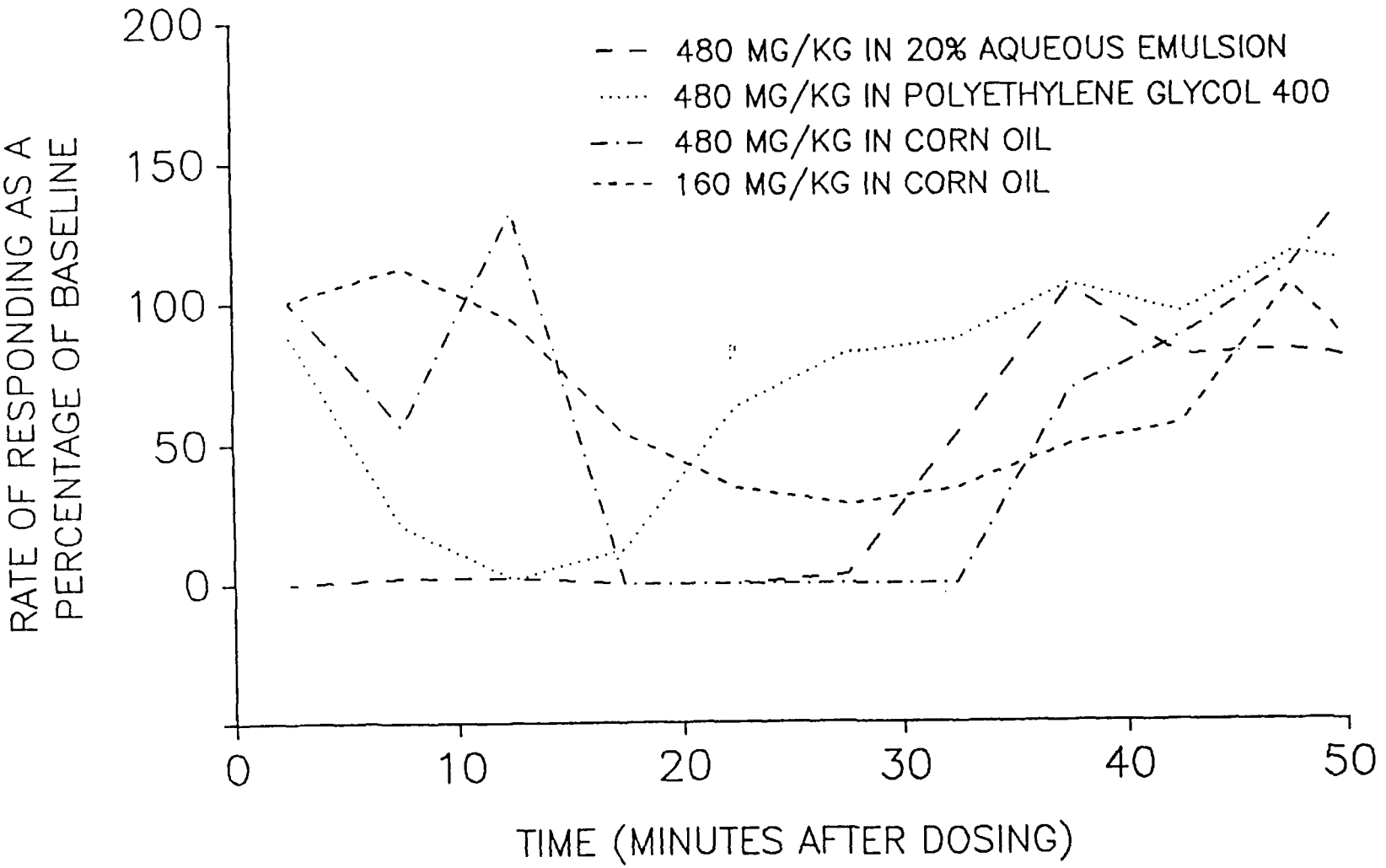


Fig. 1: Data for 1 of 4 rats who received each treatment. Response rates were calculated for each 5-minute segment of the operant session.

Fig. E-8

FIXED-RATIO RESPONDING FOLLOWING A SINGLE, ORAL BOLUS OF PCE

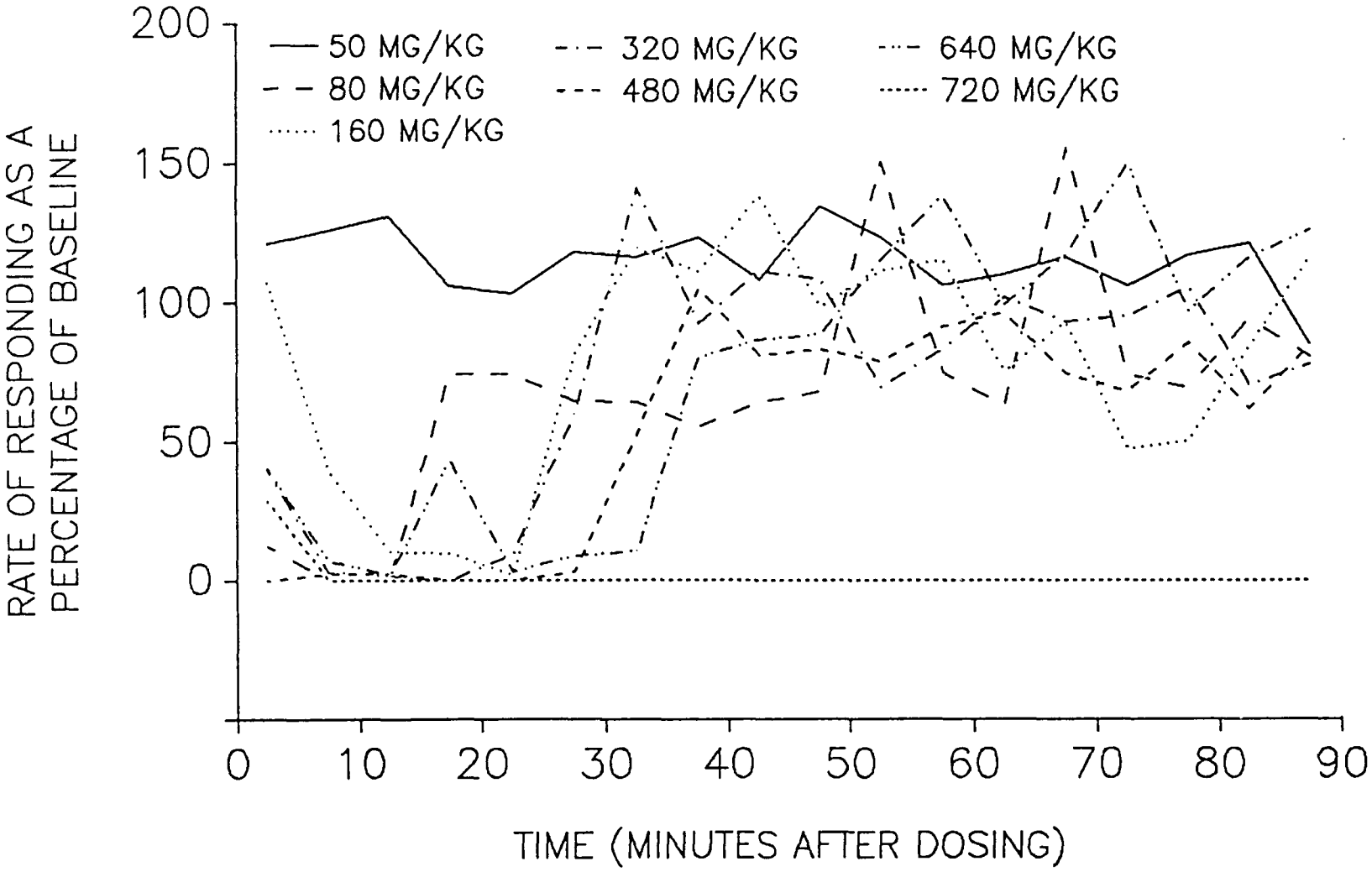


Fig. 2: Data for 1 of 4 rats who received each dose. All doses were given in a 10% aqueous Emulphor® emulsion. Response rates were calculated for each 5-minute segment of the operant session.

Fig. E-9

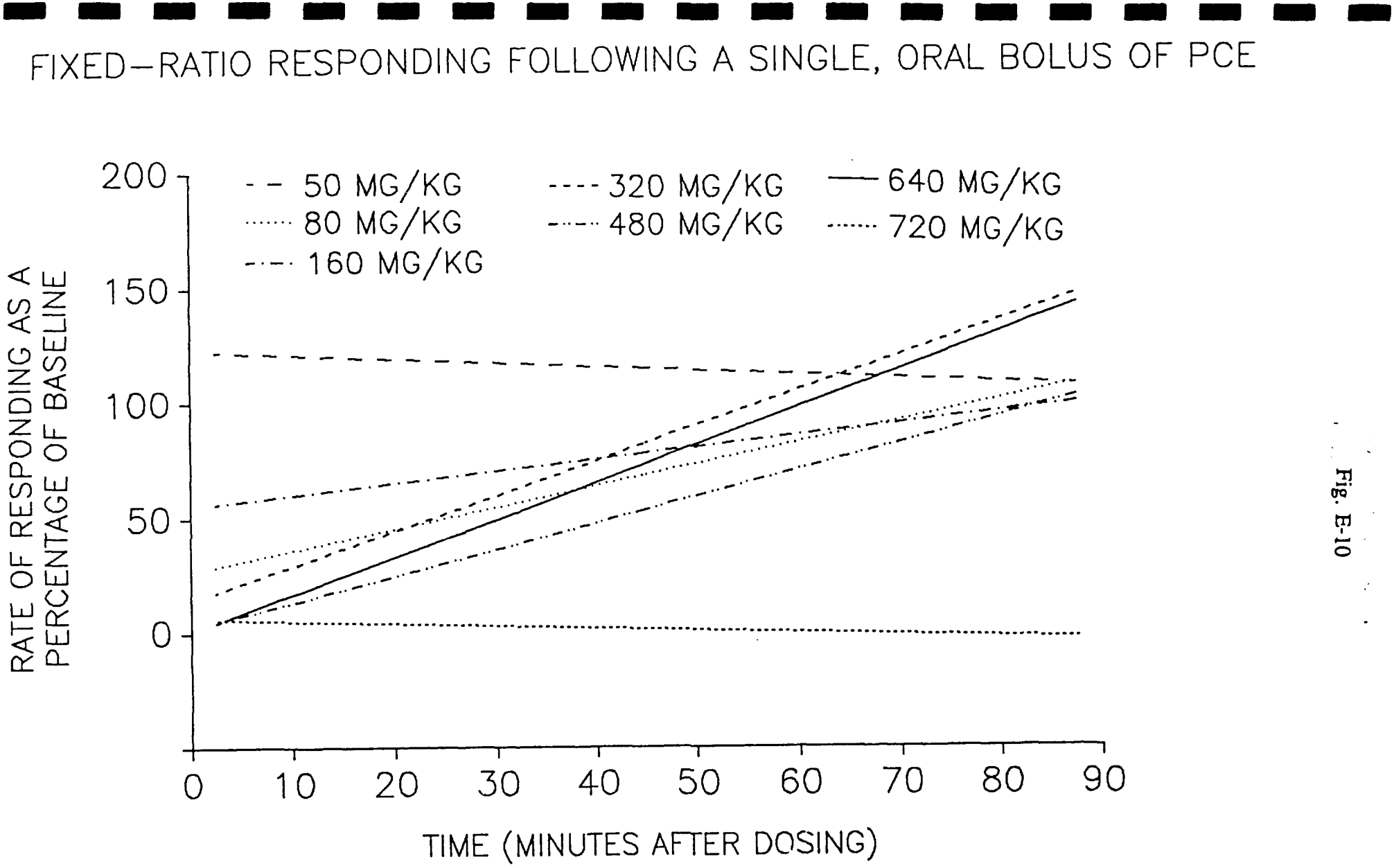


Fig. 3: Regression lines for the data presented in Fig. 2.

Fig. E-10

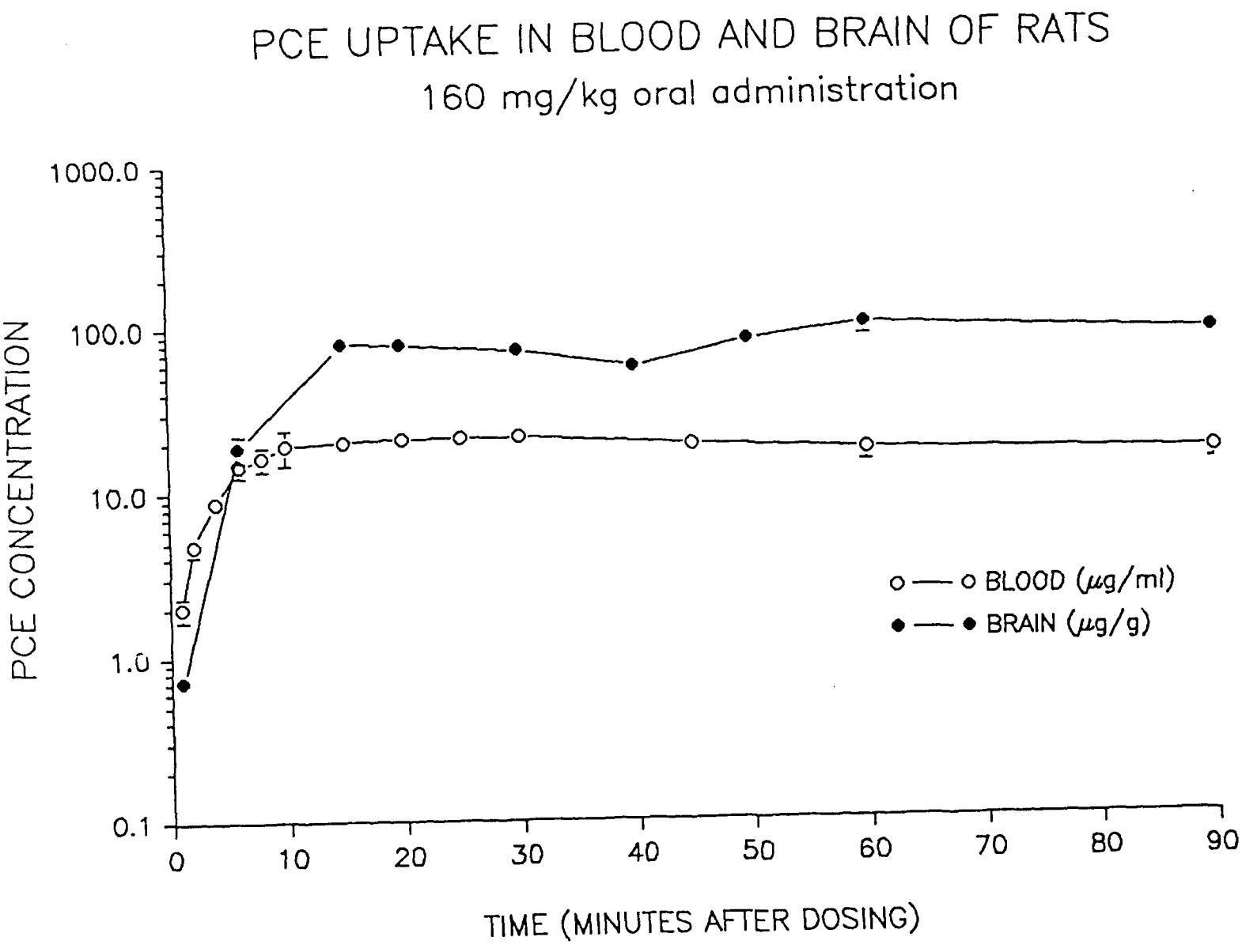


Fig. 4: Values are the mean ± SEM for 6 rats.

Fig. E-11

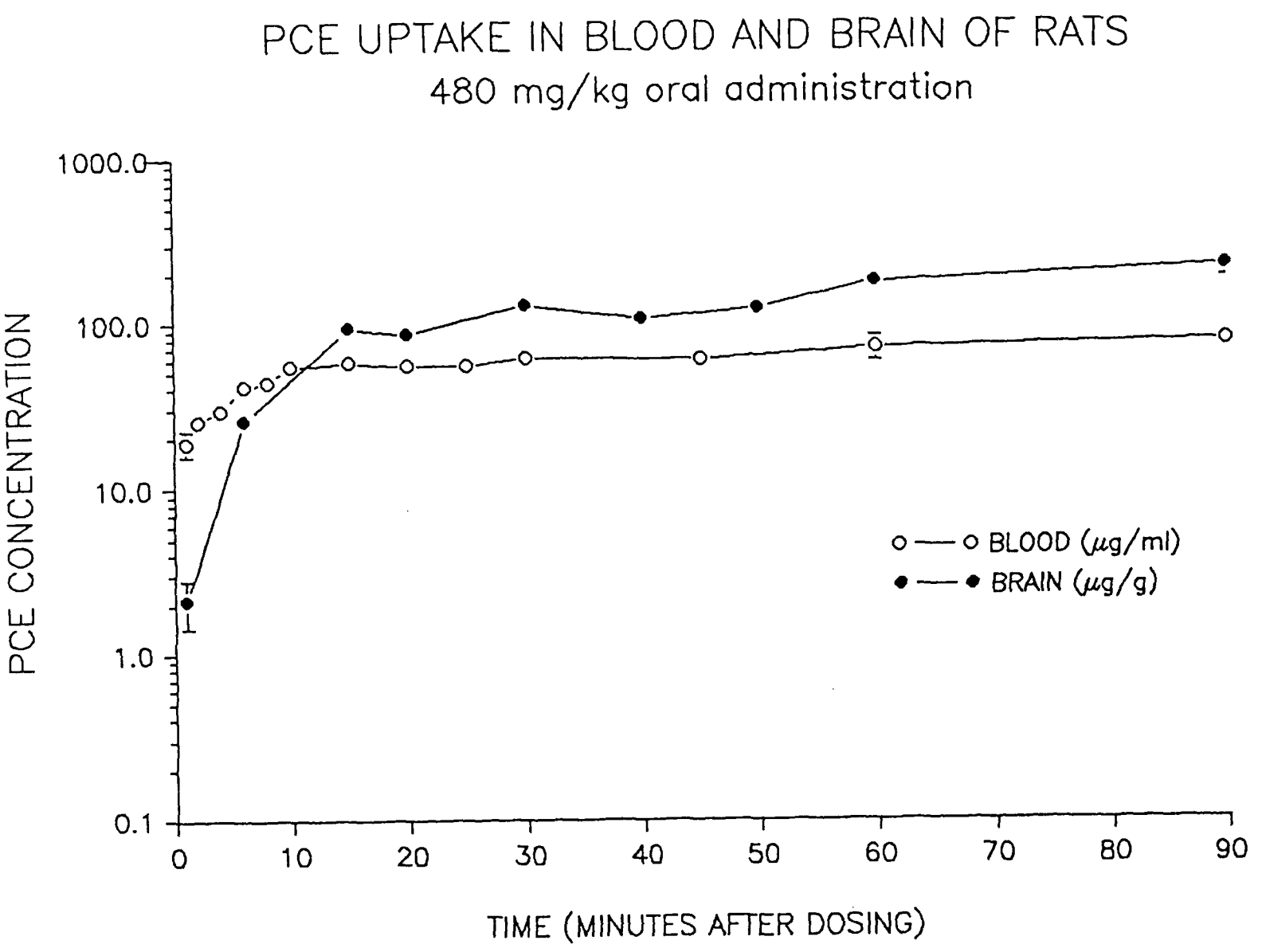


Fig. 5: Values are the mean ± SEM for 6 rats.

Fig. E-12

THE EFFECT OF TRAINING ON FIXED-RATIO RESPONDING FOLLOWING A SINGLE, ORAL BOLUS OF PCE (160 MG/KG)

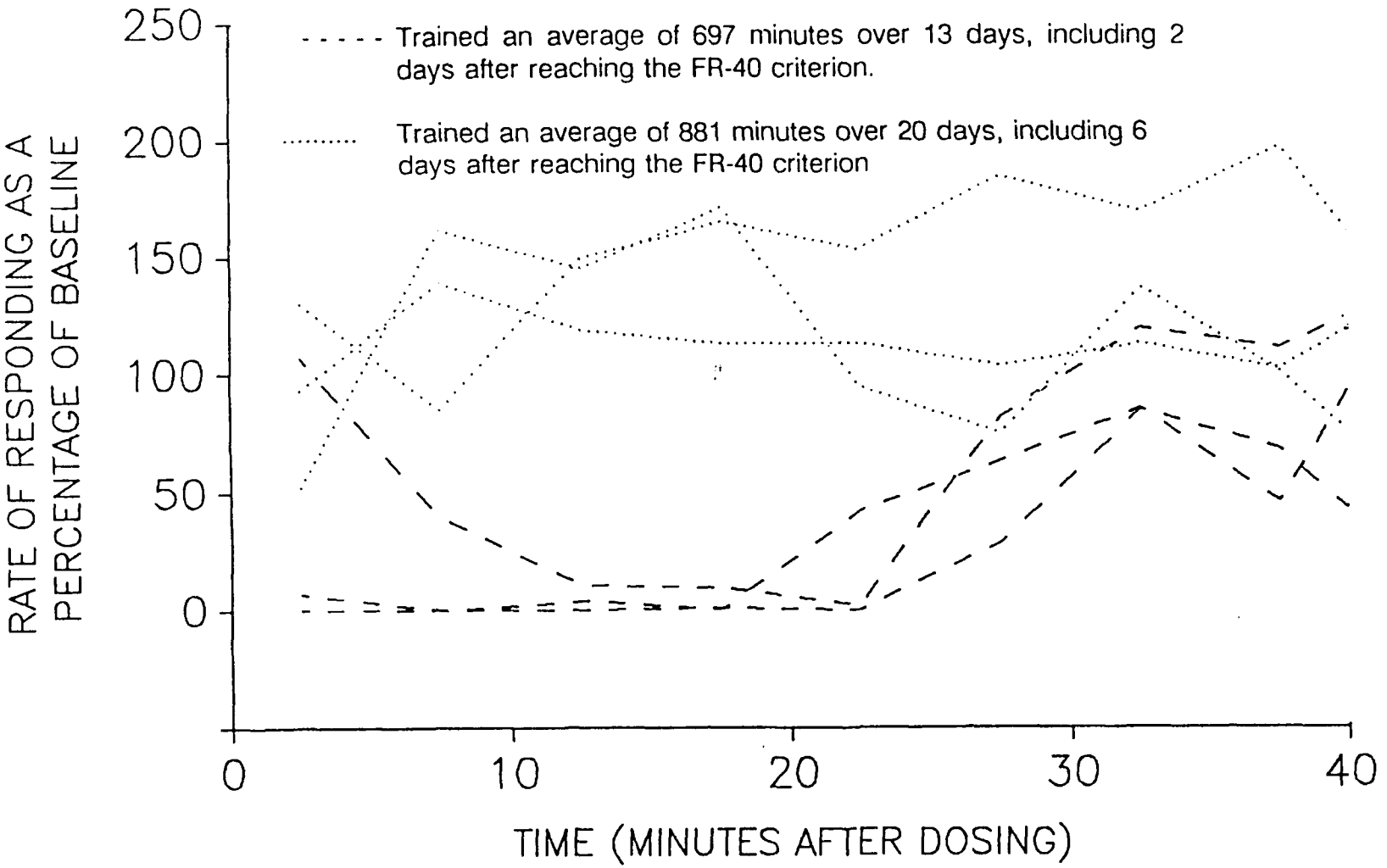


Fig. 6: All doses were given in a 10% aqueous Emulphor® emulsion. Response rates were calculated for each 5-minute segment of the operant session.

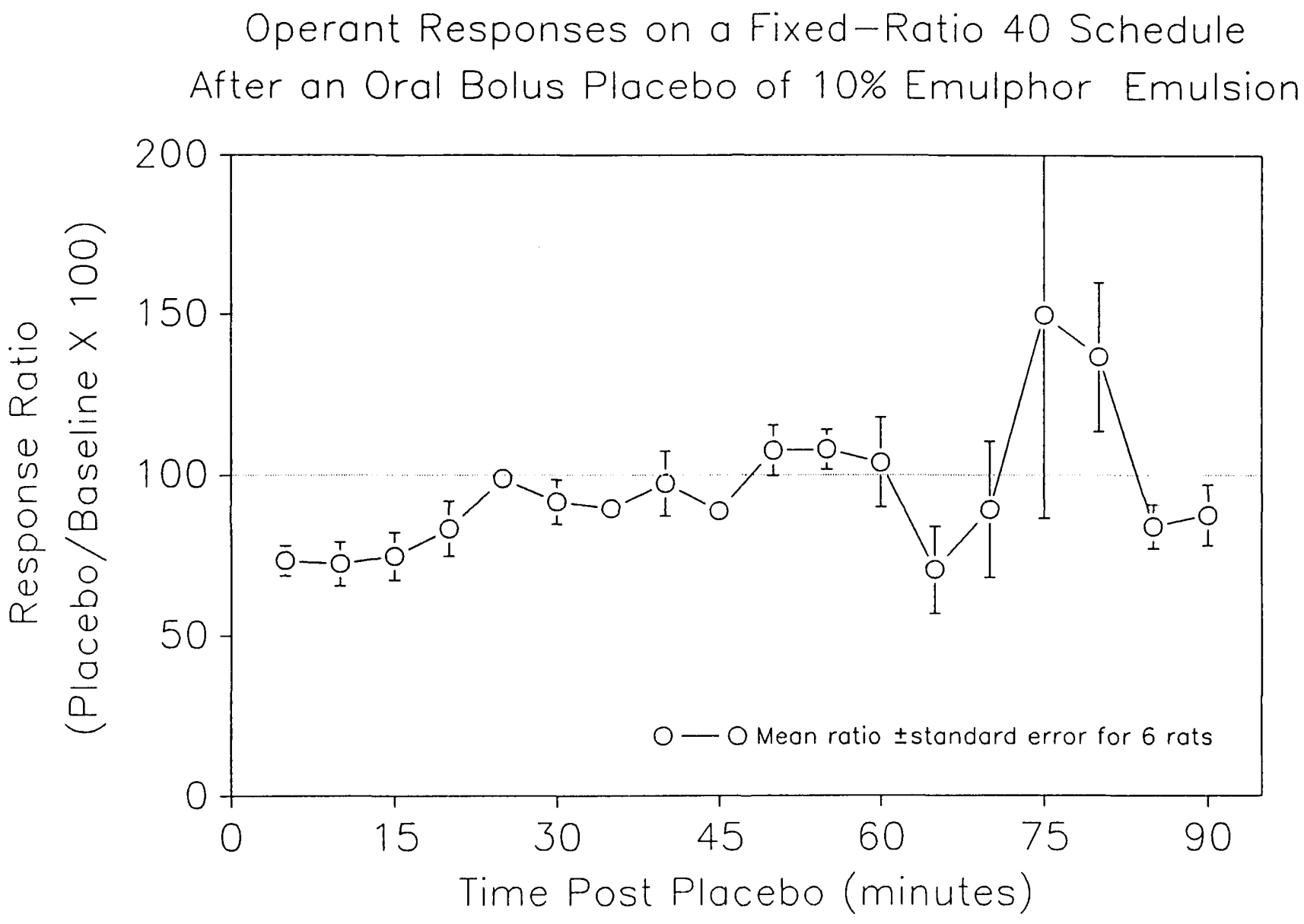

Operant Responses on a Fixed-Ratio 40 Schedule
After an Oral Bolus Placebo of 10% Emulphor Emulsion
Response Ratio
(Placebo/Baseline X 100)
200
150
100
50
0
0
15
30
45
60
75
90
Time Post Placebo (minutes)
O — O Mean ratio ±standard error for 6 rats

Figure E-14

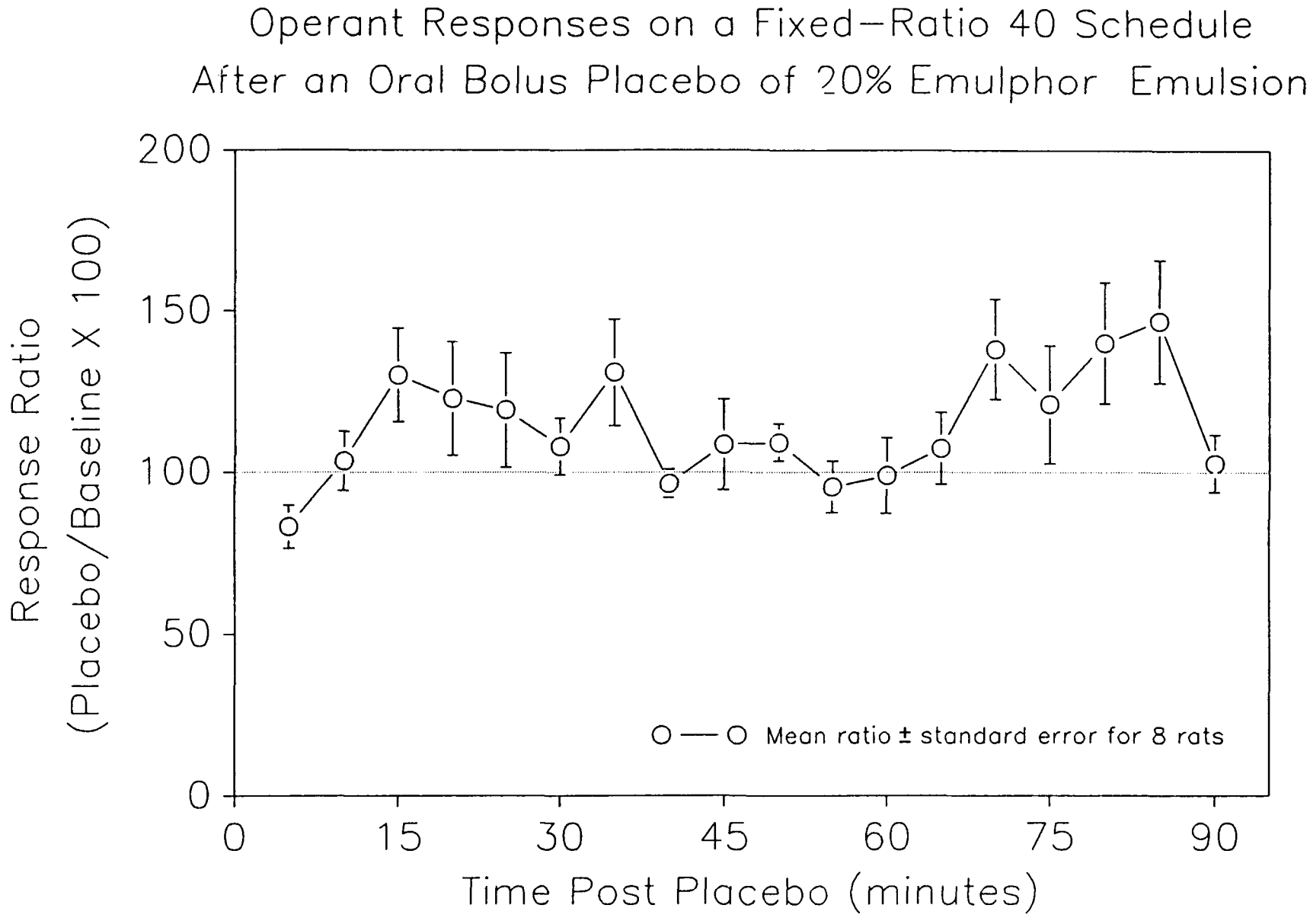

Operant Responses on a Fixed-Ratio 40 Schedule
After an Oral Bolus Placebo of 20% Emulphor Emulsion
Response Ratio
(Placebo/Baseline X 100)
200
150
100
50
0
0
15
30
45
60
75
90
Time Post Placebo (minutes)
O — O Mean ratio ± standard error for 8 rats

Figure E-15

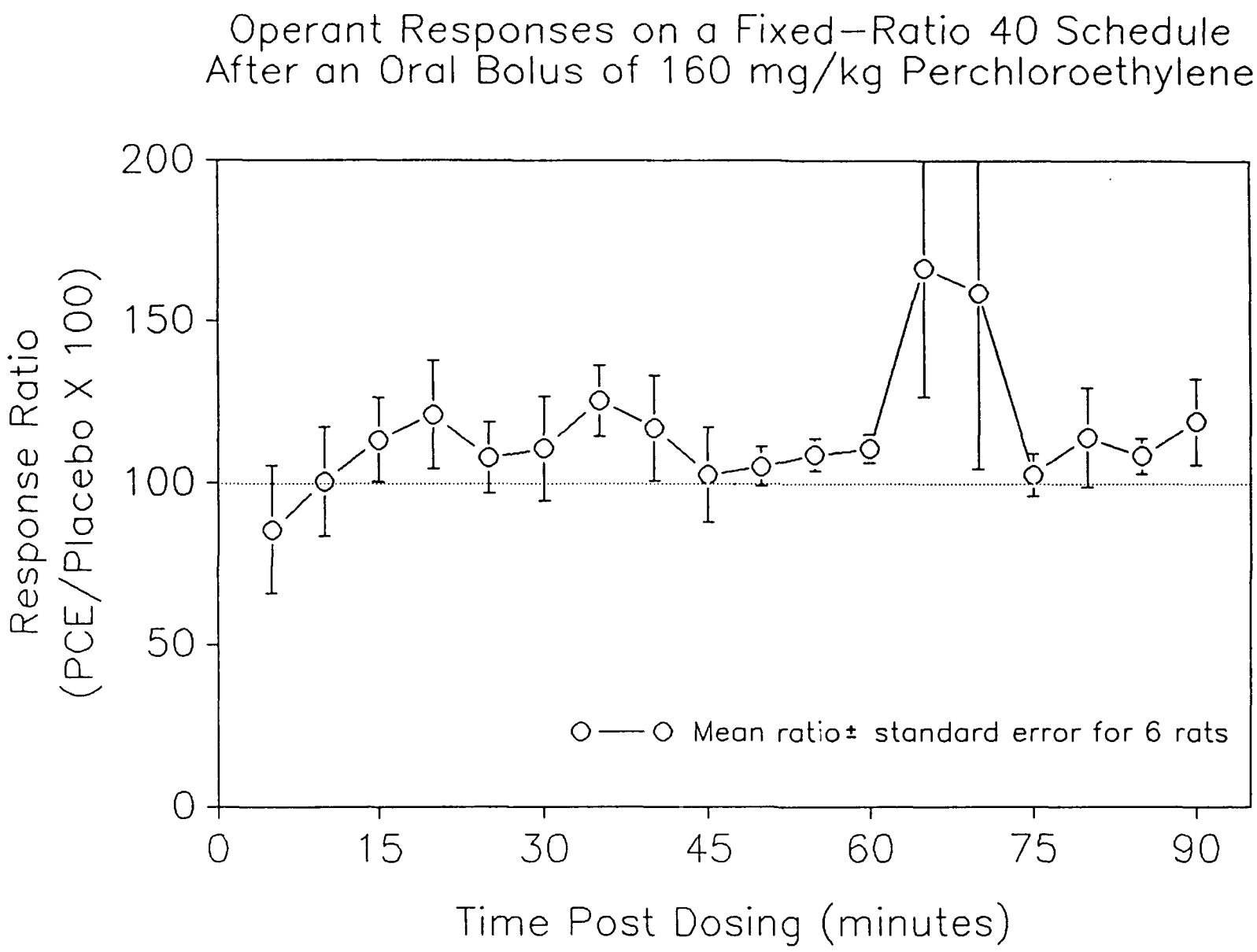

Operant Responses on a Fixed-Ratio 40 Schedule
After an Oral Bolus of 160 mg/kg Perchloroethylene
Response Ratio
(PCE/Placebo X 100)
200
150
100
50
0
0
15
30
45
60
75
90
Time Post Dosing (minutes)
Mean ratio ± standard error for 6 rats

Figure E-16

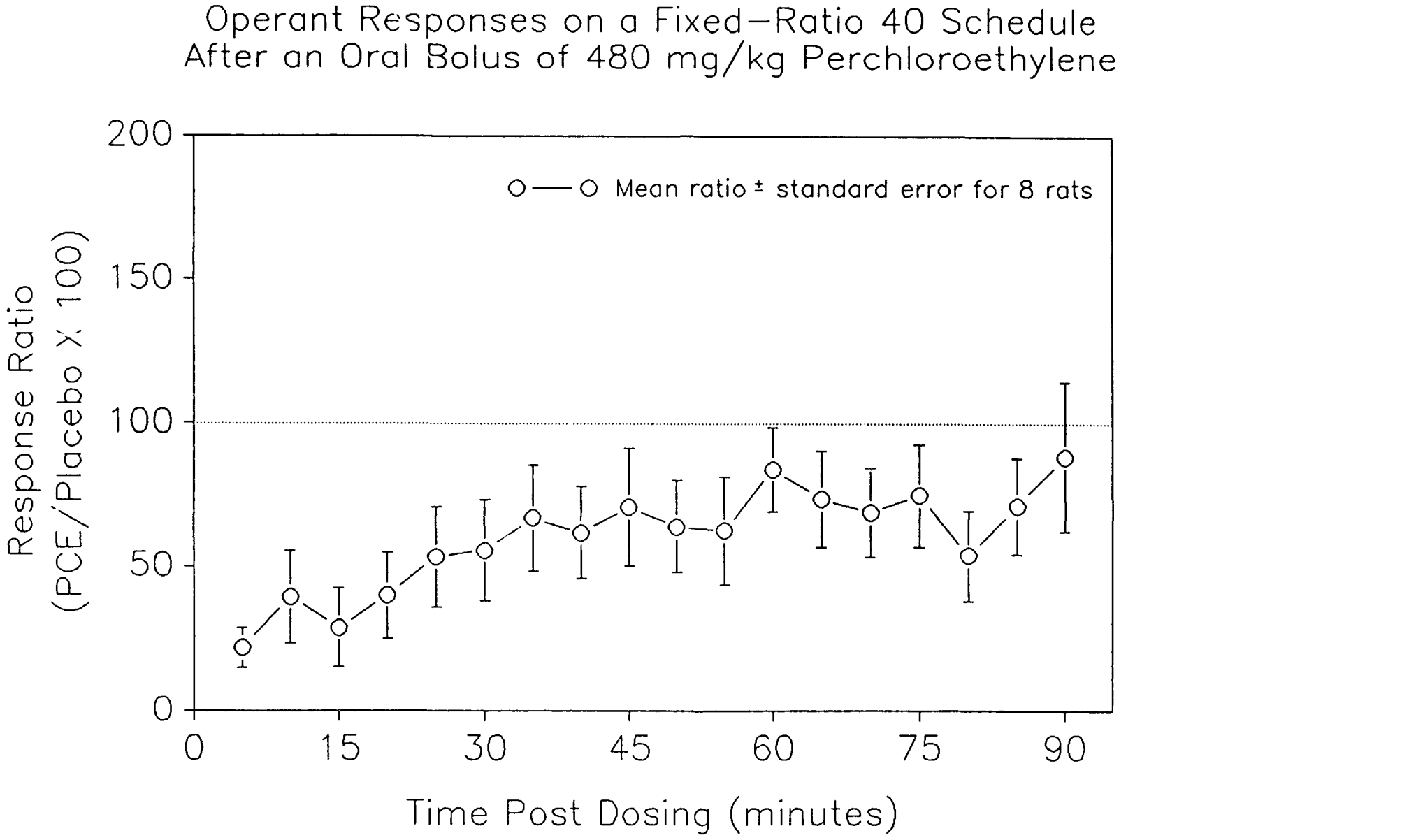

Operant Responses on a Fixed-Ratio 40 Schedule
After an Oral Bolus of 480 mg/kg Perchloroethylene
Mean ratio ± standard error for 8 rats
Response Ratio
(PCE/Placebo X 100)
200
150
100
50
0
0
15
30
45
60
75
90
Time Post Dosing (minutes)

Figure E-17

Operant Responses of One Rat on a Variable Interval 60 Schedule During Inhalation Exposures to 1,1,1—Trichloroethane

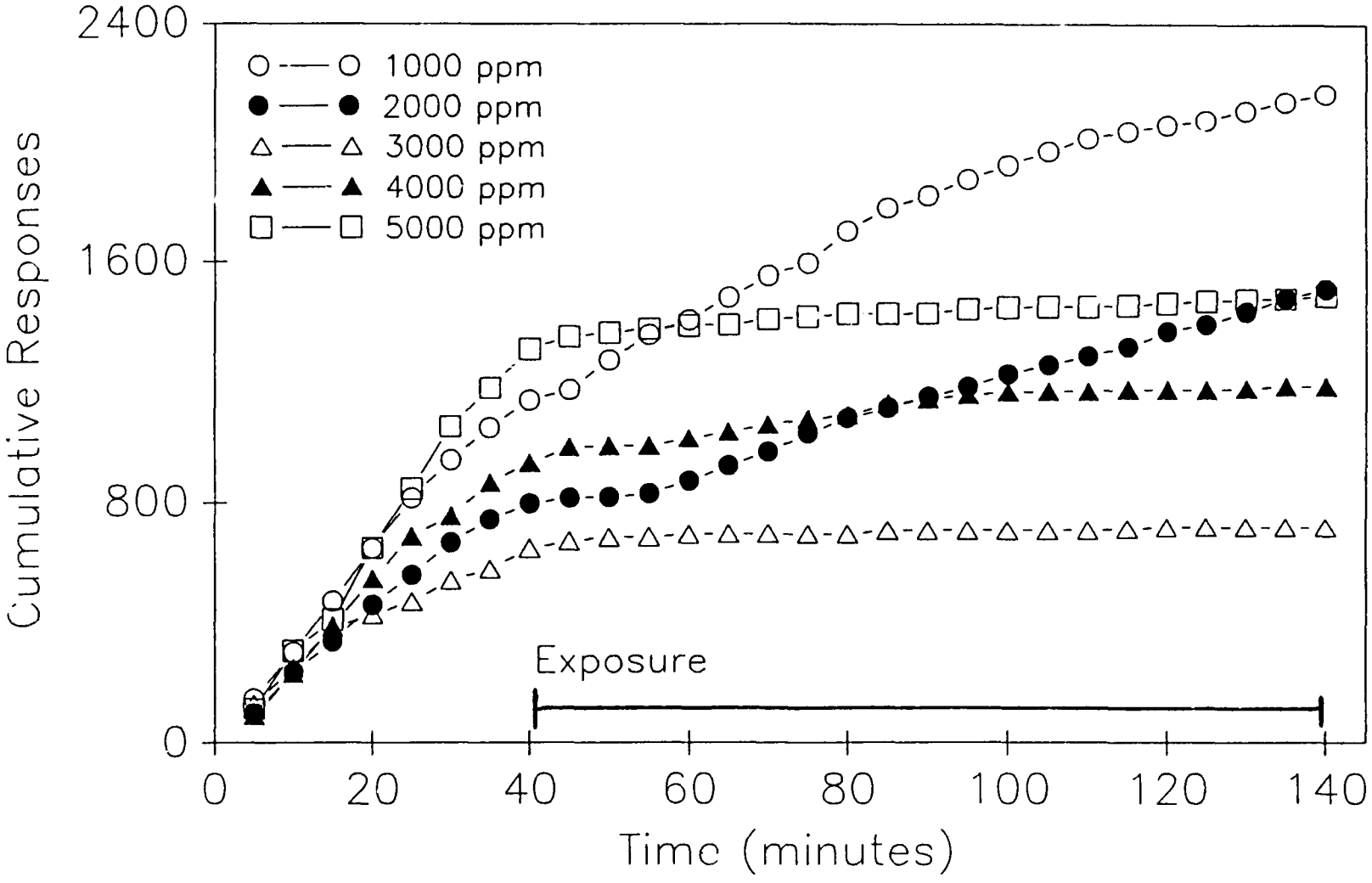


Figure E-18

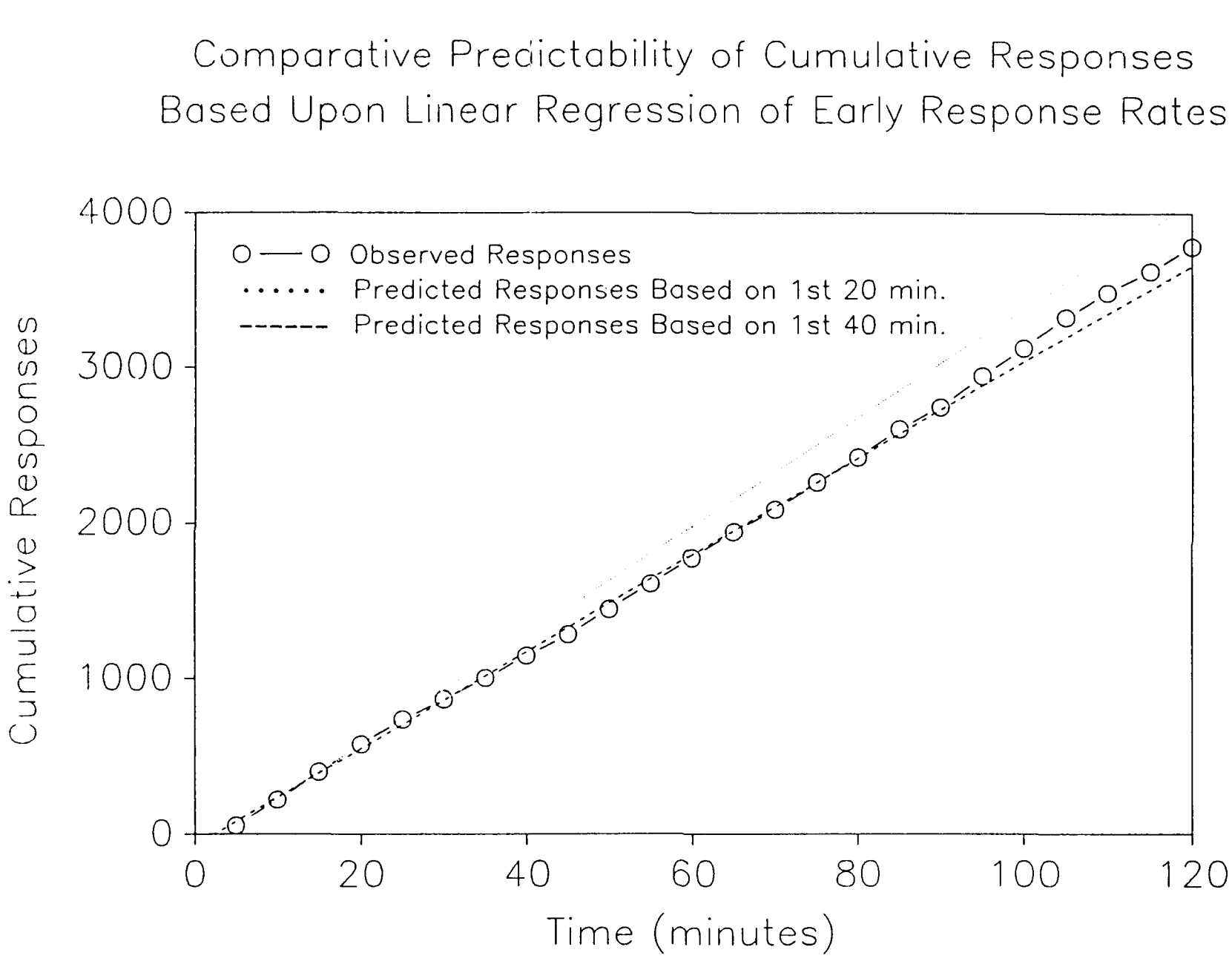

Comparative Predictability of Cumulative Responses
Based Upon Linear Regression of Early Response Rates
Observed Responses
Predicted Responses Based on 1st 20 min.
Predicted Responses Based on 1st 40 min.
Cumulative Responses
4000
3000
2000
1000
0
0
20
40
60
80
100
120
Time (minutes)

Figure E-19

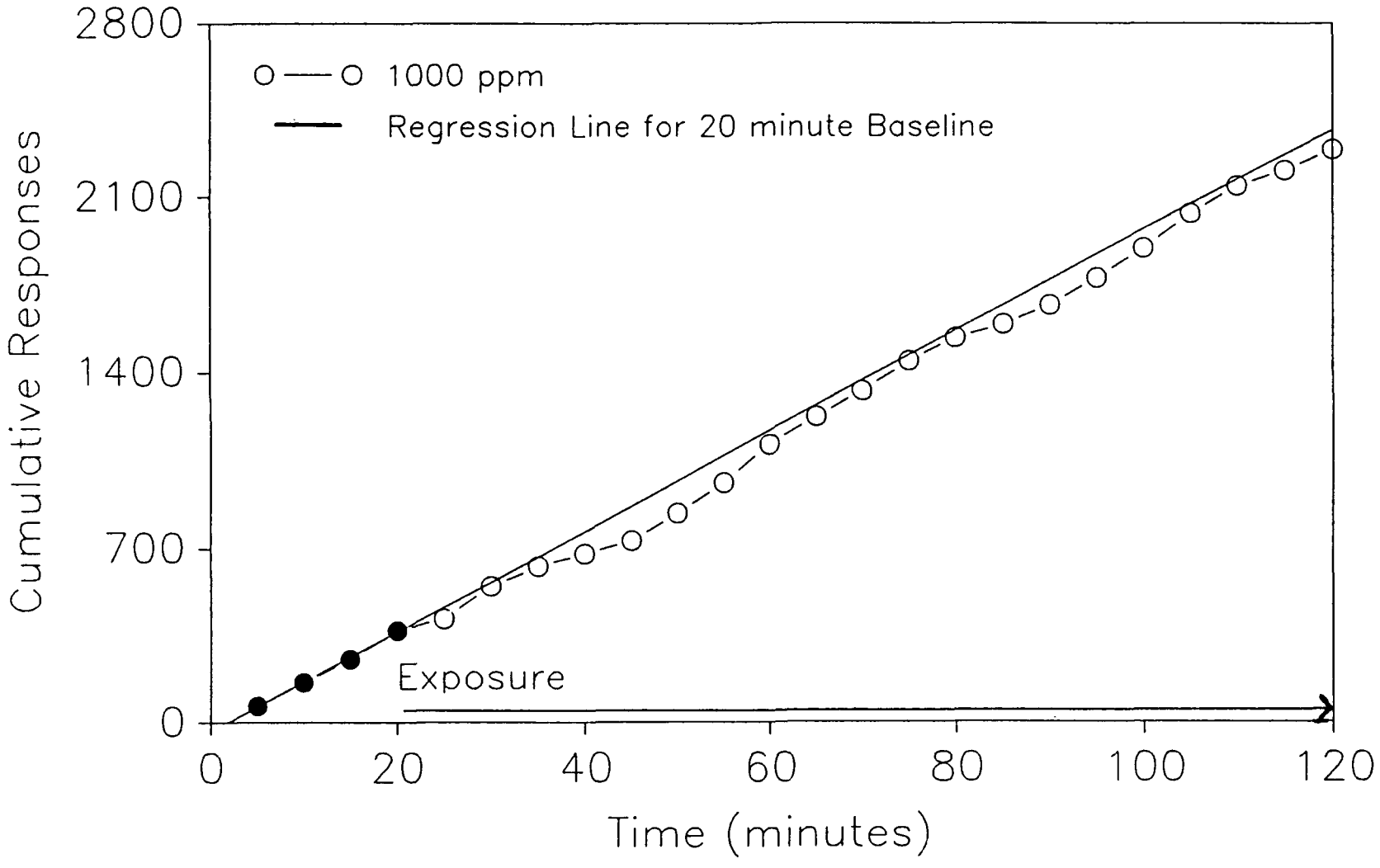

Operant Responses on a Variable Interval 30 Schedule
During a Single Inhalation Exposure to 1,1,1-Trichloroethane
Cumulative Responses
2800
2100
1400
700
0
1000 ppm
Regression Line for 20 minute Baseline
Exposure
0
20
40
60
80
100
120
Time (minutes)

Figure E-20

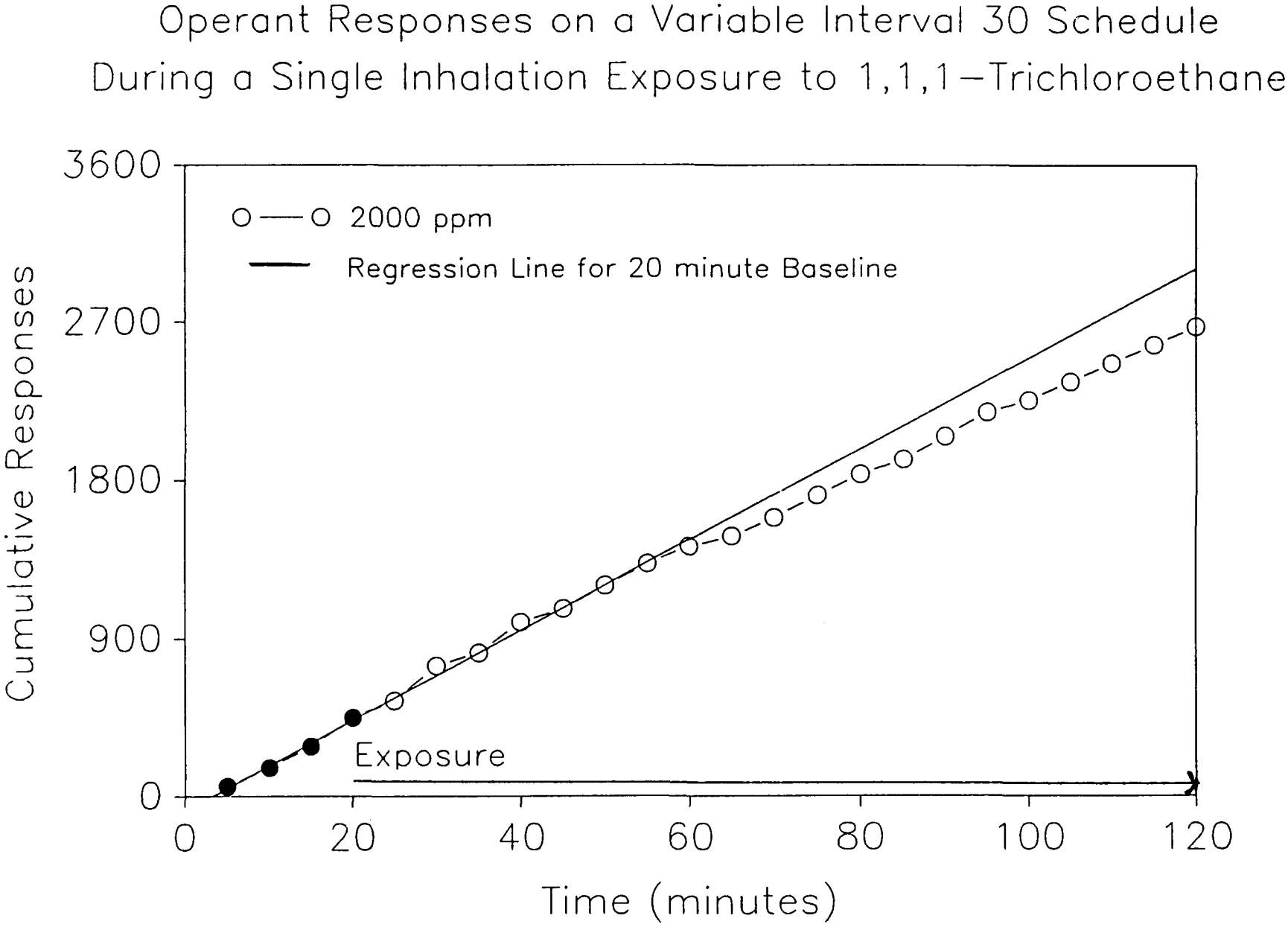

Operant Responses on a Variable Interval 30 Schedule
During a Single Inhalation Exposure to 1,1,1-Trichloroethane
3600
2700
1800
900
0
Cumulative Responses
2000 ppm
Regression Line for 20 minute Baseline
Exposure
0
20
40
60
80
100
120
Time (minutes)

Figure E-21

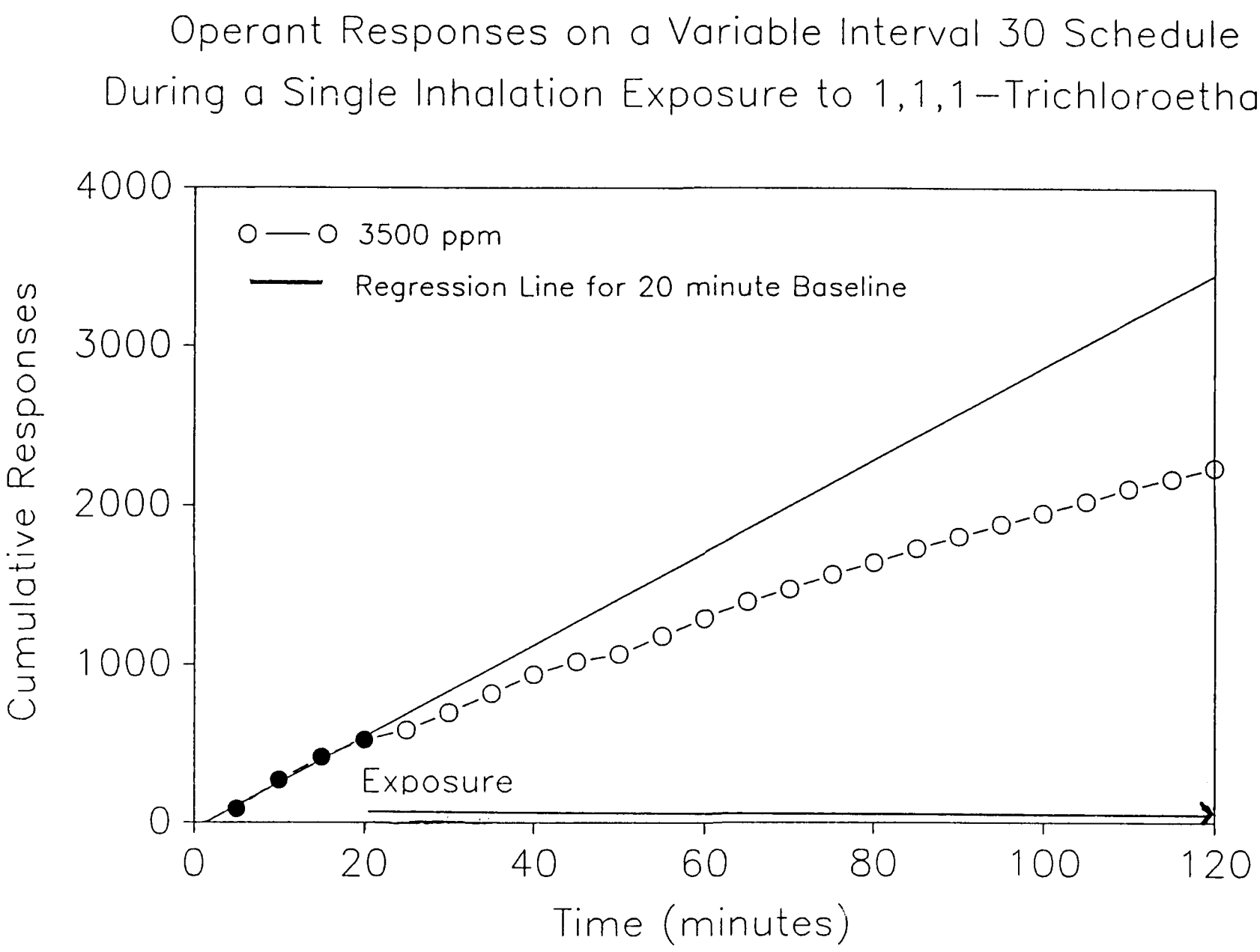

Operant Responses on a Variable Interval 30 Schedule
During a Single Inhalation Exposure to 1,1,1-Trichloroethane
Cumulative Responses
4000
3000
2000
1000
0
3500 ppm
Regression Line for 20 minute Baseline
Exposure
0
20
40
60
80
100
120
Time (minutes)

Figure E-22

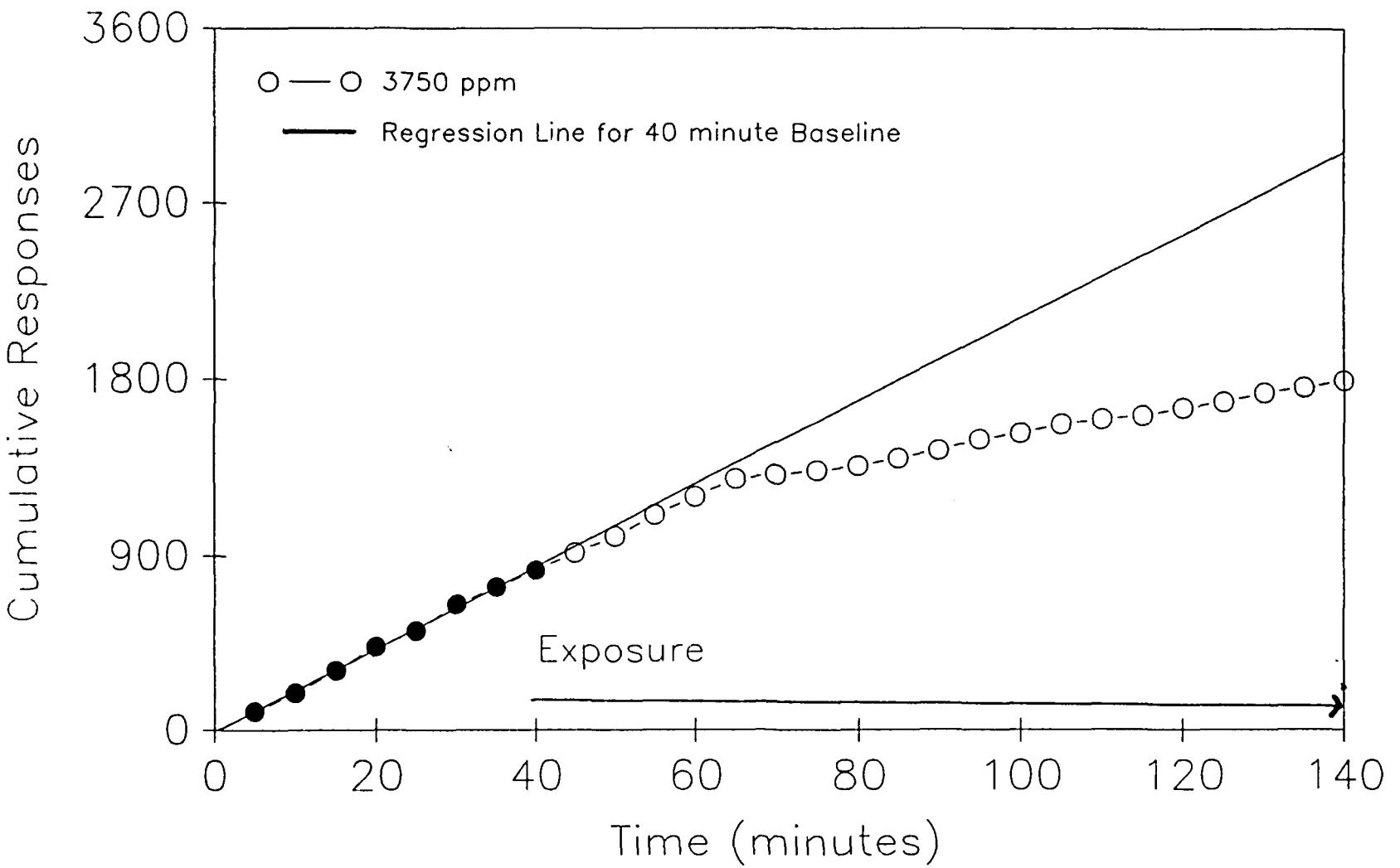

Operant Responses on a Variable Interval 30 Schedule
During a Single Inhalation Exposure to 1,1,1-Trichloroethane
3600
2700
1800
900
0
Cumulative Responses
O — O 3750 ppm
Regression Line for 40 minute Baseline
Exposure
0
20
40
60
80
100
120
140
Time (minutes)

Figure E-23

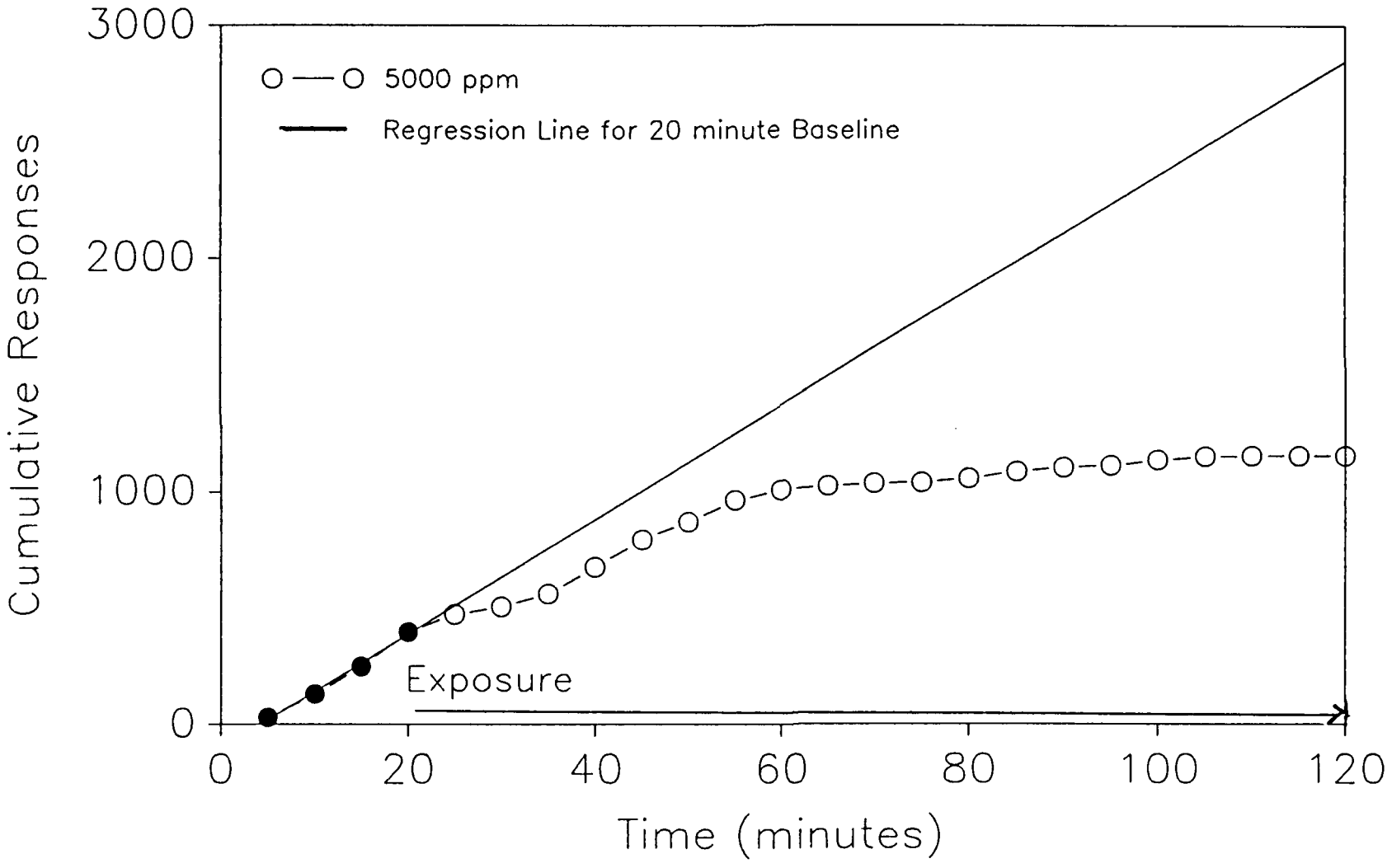

Operant Responses on a Variable Interval 30 Schedule
During a Single Inhalation Exposure to 1,1,1-Trichloroethane
Cumulative Responses
3000
2000
1000
0
O — O 5000 ppm
Regression Line for 20 minute Baseline
Exposure
0
20
40
60
80
100
120
Time (minutes)

Figure E-24

TOXICOLOGICAL INTERPRETATION OF NEUROBEHAVIORAL DATA

UNIVERSITY OF ROCHESTER MEDICAL CENTER, ROCHESTER, NY
JUNE 22-24, 1992

Day 1. A.M. Criteria for Determining Neurotoxic Potential

Chair:	Anger (U. Oregon)
Primary Papers:	MacPhail (EPA) O'Donoghue (Kodak)
Panel:	Wood (NYU) McMaster (EPA) Mattson (Dow) Daughtrey (EXXON)

Core Questions:

1. What are the criteria for determining that a chemical is a primary neurotoxicant? Must neurotoxicity predominate in the spectrum of toxic endpoints?

2. How might dose-response functions be used to deal with the problem that, at high enough doses, many chemicals inevitably will meet some neurotoxicity criteria?

3. What role should be played in the interpretation of behavioral data by identification of biological substrates such as structural changes, electrophysiology, and neurochemistry?

4. Which criteria, such as potency and irreversibility, should constitute a basis for identification as a neurotoxicant? Which criteria should provide a basis for reportable data? Should a set of categories, as with those that govern EPA cancer classification, be devised? How else should guidelines be framed?

Figure E-25

Day 1. P.M. Developmental Neurotoxicity

Chair:	Reuhl (Rutgers)
Primary Papers:	Spear (SUNY Binghamton) Wier (SmithKline)
Panel:	Cox (Rochester) Rodier (Rochester) Cranmer (Arkansas) Garman (Vet Consultants)

Core Questions:

1. How should maternal and offspring dose-response questions be treated in context of maternal toxicity? To what extent would paired feeding and cross-fostering designs be necessary?

2. What is the significance of transient outcomes, such as a 1-day delay in attainment of a developmental landmark?

3. Should neuropathological assessment be conducted at more than one age? Are repeated determinations needed?

4. Which endpoints (if any, e.g., startle) should be sampled repeatedly? Are there optimal times (excluding landmarks) for different endpoints? Which do you consider the most crucial endpoints to assay during infancy? After weaning?

5. Under what circumstances would lifetime, longitudinal assays be critical?

6. What kinds of statistical models are appropriate for conditions providing multiple endpoints at multiple ages? Are there appropriate models for litter effects?

7. EPA suggests that developmental toxicity is the most sensitive manifestation of toxicity. Does such a statement apply to neurotoxicants? If so, what is the evidence?

Figure E-26

Day 2. A.M. Activity and Observational Data

Chair:	Elsner (Zurich)
Primary Papers:	Evans (NYU) Foss (Argus)
Panels:	Moser (ManTech) Broxup (Bio-Research) Orr (Southwest) Stanton (EPA)

Core Questions:

1. Are these measures valid indicators of neurotoxicity? Under what circumstances, precautions, and restrictions? What degree of specificity should be required?

2. Can these measures alone be used to establish exposure standards for humans such as NOAELs, benchmark doses, etc.? If not, what is their function?

3. Can mechanistic conclusions be attached to such data? Is there a role for these measures in chronic studies in addition to their use in acute studies? If so, how stable are such measures over time?

Figure E-27

Day 2. P.M. Schedule-Controlled Operant Behavior I:
Identification of Neurotoxicity

Chair:	Cooper Rees (RJR)
Primary Papers:	Cory-Slechta (U. Rochester) Wenger (Arkansas)
Panel:	Gardiner (Shell) Sette (EPA) Li (Monsanto) Laties (Rochester)

Core Questions:

1. How specific is SCOB for neurotoxicity? What are some possible confounders? How might confounding be overcome?

2. Should SCOB follow or be concurrent with other potential criteria such as observational and activity measures? That is, where should it be positioned in a sequence of assessments?

3. Can SCOB be made less costly and prolonged? How do its costs and resource commitments compare to the other techniques? How might its efficiency be enhanced?

4. How are changes in response rate to be interpreted? Does a rate change invariably indicate neurotoxicity? If not, how should it be interpreted? Also, how much of a change in rate must occur to indicate an adverse effect?

5. How is it possible to distinguish performance degradation induced by neurotoxicity and that resulting from other kinds of systemic toxicity? Might the performance of sick (unmotivated?) animals be misinterpreted as neurotoxicity?

Day 3. A.M. Schedule-Controlled Operant Behavior II. Advanced Applications

Chair:	Gilbert (U. Washington)
Primary Papers:	Rice (Ottawa) Newland (Auburn U.)
Panel:	Gentry (U. Charleston) Paule (NCTR) Christoph (DuPont) Maurissen (Dow)

Core Questions:

1. Is SCOB an efficient way to evaluate questions about learning and memory? Are there differences between acute and chronic exposure questions?

2. How specifically can questions about sensory and motor function be asked with SCOB? Would you rely on such data to set standards for human exposure?

3. Compare SCOB with other approaches (such as avoidance, mazes, startle, etc.) for assessing learning, memory, sensory function, and motor performance in terms of efficiency, expense, and species comparability.

4. Are certain schedules to be preferred in assessing neurotoxicity? Are some to be preferred in the hazard detection and others in dose-response modeling phases of risk assessment?

5. What does SCOB reveal about mechanisms, if anything?

Day 3. P.M. Case Studies

Chair:	Tilson (EPA)
Primary Papers:	Isopropanol (Gill) Toluene (Wood)
General Discussion:	All Participants

Core Questions:

1. Do the animal data correspond to what is known about the effects on human performance in terms of specific functions and exposure variables?

2. What modifications, in the current approaches to neurotoxicity evaluation, might be suggested by these case studies?

Figure E-30

TOXICOLOGY AND APPLIED PHARMACOLOGY 110, 303–314 (1991)

Physiological Pharmacokinetic Modeling of Inhaled Trichloroethylene in Rats[1,2]

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Physiological Pharmacokinetic Modeling of Inhaled Trichloroethylene in Rats. DALLAS, C. E., GALLO, J. M., RAMANATHAN, R., MURALIDHARA, S., AND BRUCKNER, J. V. (1991). *Toxicol. Appl. Pharmacol.* **110**, 303–314. The pharmacokinetics of trichloroethylene (TCE) was characterized during and following inhalation exposures of male Sprague–Dawley rats. The blood and exhaled breath TCE time-course data were used to formulate and assess the accuracy of predictions of a physiologically based pharmacokinetic (PB-PK) model for TCE inhalation. Fifty or 500 ppm of TCE was inhaled by unanesthetized rats of 325–375 g for 2 hr through a miniaturized one-way breathing valve. Repetitive samples of the inhaled and exhaled breath streams, as well as arterial blood, were collected concurrently during and for 3 hr following the exposures and analyzed for TCE by headspace gas chromatography. Respiratory rates and volumes were continuously monitored and used in conjunction with the pharmacokinetic data to delineate uptake and elimination profiles. Levels of TCE in the exhaled breath attained near steady-state soon after the beginning of exposures, and were then directly proportional to the inhaled concentration. Exhaled breath levels of TCE in rats were similar in magnitude to values previously published for TCE inhalation exposures of humans. Levels of TCE in the blood of the 50 ppm-exposed animals also rapidly approached near steady-state, but blood levels in the 500 ppm-exposed animals rose progressively, reaching concentrations 25- to 30-fold higher than in the 50 ppm group during the second hour of exposure. The 10-fold increase in inhaled concentration resulted in an 8.7-fold increase in cumulative uptake, or total absorbed dose. These findings of nonlinearity indicate that metabolic saturation ensued during the 500 ppm exposure. The PB-PK model was characterized as blood flow-limited with TCE eliminated unchanged in the exhaled breath and by saturable liver metabolism. The uptake and elimination profiles were accurately simulated by the PB-PK model for both the 50 and 500 ppm TCE exposure levels. Such a model may be quite useful in risk assessments in predicting internal (i.e., systemically absorbed) doses of TCE and other volatile organics under a variety of exposure scenarios.

Trichloroethylene (1,1,2-trichloroethylene, TCE) is a volatile organic compound (VOC) which has been widely used as a surgical anesthetic, fumigant, extractant in food processing, metal degreaser, dry cleaning agent, and solvent in other commercial applications. It has been estimated that of 3.5 million persons believed to be occupationally exposed to TCE in the U.S., at least 100,000 workers are exposed full-time, and that two-thirds of these

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are in work environments where there are not adequate control measures (NIOSH, 1978). Although excessive exposures to TCE vapors have resulted in cardiac arrhythmias and in central nervous system depression, most occupational exposures do not result in apparent manifestations of toxicity (Defalque, 1965; NIOSH, 1973; U.S. EPA, 1985). There is considerable concern, however, that TCE may be a human carcinogen. TCE has been reported to produce an increased incidence of hepatocellular carcinoma in B6C3F1 mice subjected daily for their lifetime to high oral doses of the chemical (NCI, 1976; NTP, 1983). More recent studies have also shown that TCE can be carcinogenic in animals upon inhalation exposure (Fukuda *et al.*, 1983; Maltoni *et al.*, 1988).

Assessment of toxic and carcinogenic risks of exposure to TCE and other VOCs has become a subject of major importance over the last decade. Although it has formerly been common practice to calculate risk estimates on the basis of administered dose-toxicity/tumor incidence data, it is now recognized that the internal, or target organ dose is a more accurate and direct determinant of the magnitude of injury. The dose of chemical actually reaching a target organ is dependent upon kinetic processes which may vary considerably with the administered dose, route of exposure, and animal species. Thus, recognition and use of pharmacokinetic data can substantially reduce uncertainties inherent in the route-to-route, high-dose to low-dose and species-to-species extrapolations often necessary in risk assessment (Gehring *et al.*, 1976; Clewell and Andersen, 1985; NRC, 1987).

There have been a relatively large number of studies of the pharmacokinetics of TCE in humans, but data on the time-course of alveolar and blood levels during ongoing inhalation exposures are quite limited. Most human studies have focused on the elimination of TCE and its major metabolites postexposure (Stewart *et al.*, 1970; Kimmerle and Eben, 1973a; Monster *et al.*, 1976; Sato *et al.*, 1977). Additional studies to obtain TCE time-course profiles are increasingly limited by the ethical question of exposing persons to a potential human carcinogen. Thus, investigations utilizing laboratory animals must be largely relied on to provide such information.

Surprisingly, there are relatively few data available in the literature on the time-course of TCE or its metabolites in laboratory animals inhaling the chemical. Most existing studies are limited to the elimination phase following exposure (Kimmerle and Eben, 1973b; Nakajima *et al.*, 1988; Fisher *et al.*, 1989). Technical difficulties with measuring solvent uptake and respiratory functions serially in small animals during inhalation exposures have hindered accurate definition of TCE uptake and elimination profiles. Prout *et al.* (1985) did investigate the time-course of TCE and its major metabolites in the bloodstream of mice and rats given a 1,000 mg/kg oral dose of TCE in corn oil. The study results are useful qualitatively in that they reveal that TCE undergoes much more extensive first-pass metabolism in the mouse than in the rat. The results are of limited use quantitatively, however, in that blood was collected from only one animal at each time-point. Balance studies in mice and rats administered [^{14}C]TCE orally (Prout *et al.*, 1985; Dekant *et al.*, 1986) and by inhalation (Stott *et al.*, 1982) confirm that mice have a higher TCE metabolic capacity than do rats. In each study, ^{14}C levels in animal tissues were measured only at a single time (i.e., 50 or 72 hr) postexposure. Thus, blood and tissue TCE concentration versus time data that are presently available are not adequate to delineate the internal dose of TCE received during inhalation exposures.

Physiologically based pharmacokinetic (PB-PK) models have been formulated for a number of VOCs, in an effort to better understand and forecast the dynamics of the chemicals in the blood and tissues of laboratory animals and humans. The NRC (1986) was the first to describe the use of a PB-PK model for TCE in route-to-route and rat-to-human extrapolations. Bogen (1988) applied the styrene PB-PK model of Ramsey and Andersen (1984) to

predict relationships between the administered dose of TCE, the toxicologically effective dose, and the risk of cancer in humans. Experimental data were not supplied in either case, however, to test the fidelity of the TCE model predictions. Fisher *et al.* (1989) recently developed a PB-PK model to describe the dynamics of TCE and trichloroacetic acid in pregnant rats exposed to TCE by inhalation and ingestion. The model simulations compared favorably with the limited blood TCE concentration time data which were available to the investigators.

In consideration of the foregoing, the objectives of the present investigation were to: (a) quantify the rate and magnitude of TCE uptake and elimination over time during the course of TCE inhalation exposures of rats, (b) accurately define blood and exhaled breath TCE concentration versus time profiles during and after the exposures, and (c) formulate a PB-PK model for inhalation of TCE based on the observed time-course data.

MATERIALS AND METHODS

Animals. Adult male Sprague–Dawley rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The animals were maintained on a constant light–dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. There were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow were provided *ad libitum*. The rats were used after at least a 14-day acclimation period, at which time their body weight ranged from 325–375 g. TCE exposures were initiated at approximately the same time each day (1000 to 1200 hr).

Test material. Trichloroethylene (TCE), of >99.99% purity, was obtained from J. T. Baker Chemical Co. (Phillipsburg, NJ). The purity of the solvent was verified by gas chromatography.

Animal preparation. All rats were surgically implanted with an indwelling carotid artery cannula, which exited the animal at the back of the neck. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of ketamine HCl (100 mg/ml), acepromazine maleate (10 mg/ml), xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v:v:v). The cannulated animals were maintained in a harness and pulley system that allowed relative freedom of movement in metabolism cages during a 24-hr recovery period after surgery.

Inhalation exposures. A known concentration of TCE was generated within a 70-liter gas sampling bag (Calibrated Instruments, Ardsley, NY) by injecting the appropriate quantity of TCE into the bag filled with air. Uniform dispersion of the vapor was insured by a magnetic stirring bar within the bag. The bag was then connected in series by Teflon tubing with a pneumotachograph, a miniaturized one-way breathing valve (Hans Rudolph, Inc., St. Louis, MO), and an empty 70-liter gas collection bag. The latter bag served as a reservoir to collect exhaled gas. The breathing valve was attached to a face mask designed to fit the rat, so that the valve entry port was directly adjacent to the nares of the test animal. Thus, separate and distinct airways for the inhaled and exhaled breath streams were established. Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. Each cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneva, Switzerland). The face mask was held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. This basic inhalation exposure setup has been illustrated previously by Dallas *et al.* (1989). TCE exposures were initiated only after stable breathing patterns were established during a 1-hr acclimation period. During the 2 hr of TCE exposure and for 3 hr afterward, serial inhaled and exhaled breath samples were taken at approximately the same time as blood samples from the carotid artery cannula. The blood samples were then analyzed for TCE content by headspace gas chromatography, whereas the air samples were injected directly into the gas chromatograph.

Respiratory measurements and calculations. The respiration of each animal was continuously monitored according to previously published methods (Dallas *et al.*, 1983, 1986). The airflow created by the animal's inspiration was recorded both during and following TCE inhalation exposure in terms of minute volume (volume of respiration per minute, or V_E), respiratory rate (f), and tidal volume (V_R). Mean values for these parameters were obtained by averaging the measurements taken at 10-min intervals in individual animals during the 2-hr exposures. Mean ± SE values for the 500 ppm-exposure group (n = 6) were: V_E = 218.0 ± 20.2 ml/min, f = 128.4 ± 7.1 breaths/min, and V_T = 1.71 ± 0.15 ml. Means ± SE for the 50 ppm group (n = 6) were: V_E = 268.9 ± 15.5 ml/min, f = 132.0 ± 7.3 breaths/min, and V_T = 2.12 ± 0.20 ml.

Calculations of TCE uptake and elimination were conducted utilizing the equations presented in a previous VOC inhalation study in rats (Dallas *et al.*, 1989). Since the V_E and the exhaled breath TCE concentration at each sampling point were measured, subtraction of the quantity of TCE exhaled by the animal from the amount inhaled yielded an estimation of the quantity of TCE taken up during sequential sampling periods (cumulative uptake). The percentage uptake during each exposure period was determined by dividing the cumulative uptake by the total inhaled dose for that time period. The statistical signifi-

cance of differences between the 50 and 500 ppm animals in percentage uptake at each time point was assessed by an unpaired t test, with $p < 0.05$ chosen at the minimum level of significance.

PB-PK model. A PB-PK model was used to describe the disposition of TCE in the rat (Fig. 1). It was assumed that a blood-flow-limited model was adequate to characterize the tissue distribution of TCE. Previous PB-PK models for VOCs have utilized blood-flow-limited organ representations (Ramsey and Andersen, 1984; Angelo and Pritchard, 1984; Dallas *et al.*, 1989). Compartmental volumes and organ blood flows were obtained from the values used by Ramsey and Andersen (1984) for rats, and scaled to 340 g, the mean body weight of rats utilized in the present study. Tissue:blood partition coefficients that characterize the extent of tissue TCE uptake were obtained from Andersen *et al.* (1987). The Michelis–Menton parameters, V_{max} and K_m, describing the rate of TCE metabolism, were initially estimated from Andersen *et al.* (1987), and were $K_m = 0.25$ μg/ml and $V_{max} = 183.3$ μg/kg/min. When scaled to the 340-g rat used in the current study, $V_{max} = 82.0$ μg/min. The final value of V_{max}, set equal to 75.0 μg/min, provided good agreement between observed and predicted blood TCE concentrations. Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport of TCE in the rat as depicted in Fig. 1, were numerically solved with the ACSL (Advanced Continuous Simulation Language) computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted blood and exhaled breath TCE concentrations as a function of time.

Analysis of TCE in air and blood. The concentration of TCE in the inhaled and exhaled air was measured with a Tracor MT560 gas chromatograph (Tracor Instruments, Austin, TX). Standards were prepared in each of four 9-liter bottles equipped with Teflon stoppers with needles from which air samples could be taken by syringe. Analyses for the 500 ppm exposures were conducted using a flame ionization detector (FID), while the analyses for the 50 ppm inhalation exposures were conducted using an electron capture detector (ECD). The detection limits for TCE in air by FID and ECD were 0.5 and 0.003 μg/ml, respectively. The ECD detector was employed for the 50 ppm exposures, since most of the postexposure exhaled breath concentrations in these animals were below the FID detection limit. In either case, air samples were procured with a gas-tight, 1-ml syringe and injected directly onto

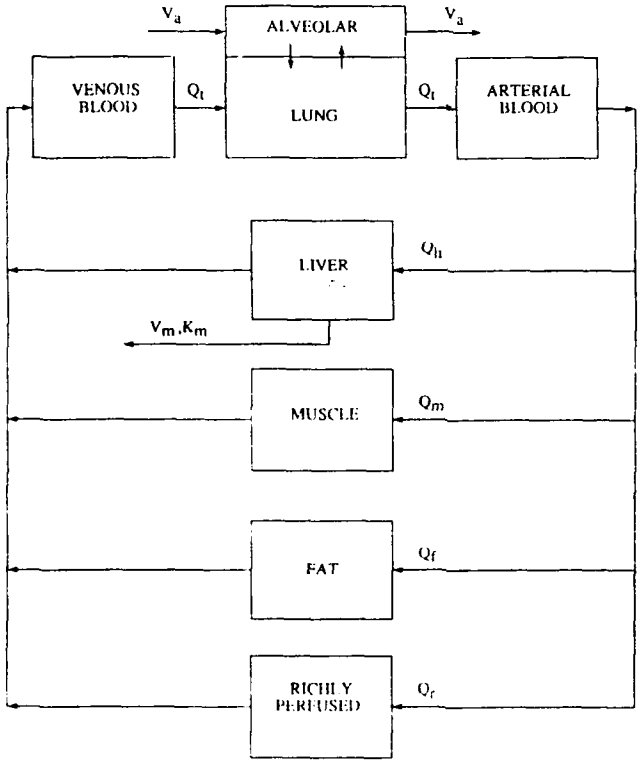

FIG. 1. Diagram of the physiological pharmacokinetic model used to simulate the uptake and elimination of inhaled TCE. The symbols and parameters used to describe the model are included in Table 1.

TABLE 1

PARAMETERS FOR THE PHYSIOLOGICAL PHARMACOKINETIC MODEL OF INHALED TCE IN THE RAT (340 g)

Parameter	Value
Alveolar ventilation rate (ml/min), VR_a	134.5 (50 ppm exposure) 109 (500 ppm exposure)
Inhaled gas concentration (μg/ml)	0.272 (50 ppm exposure) 2.69 (500 ppm exposure)
Alveolar mass transfer coefficient	500 ml/min
Blood flows (ml/min)	
Cardiac output, Q_c	106.4
Fat, Q_f	9.4
Liver, Q_l	39.8
Muscle, Q_m	12.8
Richly Perfused, Q_r	44.4
Tissue volumes (ml)	
Alveolar	2.0
Blood	25.4
Fat	30.5
Liver	13.6
Lung	3.97
Muscle	248.0
Richly Perfused	15.2
Partition coefficients	
Blood:Air	21.9
Fat:Blood	25.3
Liver:Blood	1.24
Muscle:Blood	0.46
Richly Perfused:Blood	1.24
Metabolism Constants	
V_{max} (μg/min)	75.0
K_m (μg/ml)	0.25

an 8-ft × ⅛-in. stainless-steel column packed with 0.1% XE 1000 on GraphPak. Operating temperatures were 150°C, injection port; 200°C, FID detector; 350°C, ECD detector; and 110°C, isothermal column operation. When using the ECD, gas flow rates of 40 ml/min were employed for nitrogen (carrier gas), with an additional make-up gas flow rate to the detector of 30 ml/min.

TCE levels in the blood were measured by gas chromatographic headspace analysis. Blood samples were withdrawn from the arterial cannula via a stopcock into a 1-ml syringe. Depending on the anticipated blood TCE concentration, from 25 to 200 μl of the blood was taken from the stopcock with an Eppendorf pipet and transferred to chilled headspace vials (Perkin-Elmer, Norwalk, CT). These vials were capped immediately with PTFE-lined butyl rubber septa and washers and tightly crimped. Each sample vial was then placed into the HS-6 auto-sampler unit of a SIGMA 300 gas chromatograph (Perkin-Elmer, Norwalk, CT), where it was heated to a preset temperature by a high precision thermostat device. A predetermined volume of the vapor was then injected automatically into the column for analysis. Standard curves were generated on each day that measurements were conducted by injection of known quantities of TCE into headspace vials for subsequent analysis. The column used was an 8-ft × ⅛-in. stainless-steel column packed with FFAP chromasorb W-AW (80–100 mesh). Operating temperatures were 250°C, injection port; 350°C, ECD detector; and 80°C column oven. The carrier gas was 5% argon–methane, at a flow rate of 40 ml/min, with a make-up gas flow rate to the detector of 20 ml/min.

RESULTS

While 50 and 500 ppm were the target TCE inhalation concentrations, the actual concentrations inhaled by the animals were determined by analysis of air samples taken from the airway immediately adjacent to the breathing valve. Mean (±SE) inhaled TCE concentrations for the six rats in each group were 499.8 ± 12.7 ppm for the 500 ppm exposures and 50.7 ± 0.8 ppm for the 50 ppm exposures.

The time-courses of TCE concentrations in the exhaled breath and arterial blood were delineated during and for 3 hr following 2-hr exposures of rats to 50 (Fig. 2) and 500 ppm (Fig. 3) TCE. TCE was readily absorbed from the lungs into the arterial circulation, as reflected by relatively high blood levels at the initial sampling time (i.e., 1 min). The concentration of TCE increased rapidly in the blood of the 50 ppm animals, reaching near steady-state levels within approximately 25 min. In contrast, blood levels in the 500 ppm animals increased steadily, but did not reach steady-state after 2 hr of TCE inhalation. The arterial concentrations were not proportional to the inhaled concentrations. Blood levels during the latter hour of exposure were 25–30 times higher in the 500 than in the 50 ppm group. Exhaled breath levels of TCE increased more rapidly than did blood levels after the initiation of exposures, the former attaining near steady-state within 10–15 min. The exhaled breath TCE concentrations remained

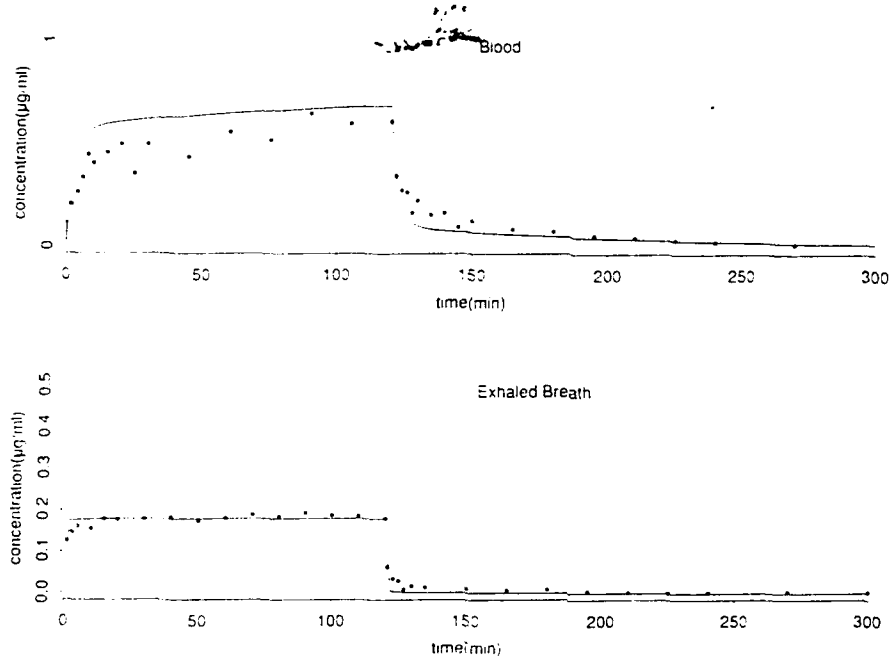

FIG. 2. Observed (●) and model-predicted (—) TCE concentrations in the arterial blood and exhaled breath of rats during and following a 2-hr, 50 ppm inhalation exposure. Each point represents the mean value for six rats.

relatively constant for the 2-hr duration in both exposure groups. Unlike blood levels, the exhaled breath levels at near steady-state were directly proportional to the inhaled concentrations. Mean TCE concentrations in the expired air during the last 1.5 hr of the 2-hr exposures were 34.6 ± 1.1 and 340.8 ± 10.6 ppm ($\bar{x} \pm$ SE, $n = 6$) in the 50 and 500 ppm groups, respectively.

The disappearance of TCE from the blood generally paralleled that in the expired air postexposure, though some disparity was observed. Concentrations of TCE measured in the exhaled breath and blood initially decreased very rapidly after exposures ceased. As can be seen in Figs. 2 and 3, the patterns of elimination differed, in that blood levels diminished more slowly than exhaled breath levels during the first 30 to 45 min postexposure. This difference was most pronounced in the 500 ppm group. Thereafter, TCE was eliminated from the blood and breath at comparable rates. The TCE levels were not monitored long enough postexposure to accurately define the terminal elimination half-lives.

The PB-PK model accurately described the uptake and elimination of TCE in both the blood and expired air. Model-generated exhaled breath and blood TCE concentrations are represented by solid lines in Figs. 2 and 3. The predictions of exhaled breath levels during inhalation were in close agreement with the direct measurements of expired TCE at both exposure levels. Postexposure exhaled breath concentrations were well simulated for the 50 ppm group, and only slightly underpredicted during the first 45 min for the 500 ppm group. The progressive increase in blood concentration during the 2-hr, 500 ppm exposure was accurately forecast by the model. The pattern of uptake of TCE into the blood of the 50 ppm animals was adequately described, although the TCE concentrations were slightly overpredicted (i.e., by about 0.1 μg/ml). The model predicted a more rapid postexposure decline in blood levels than was observed during the

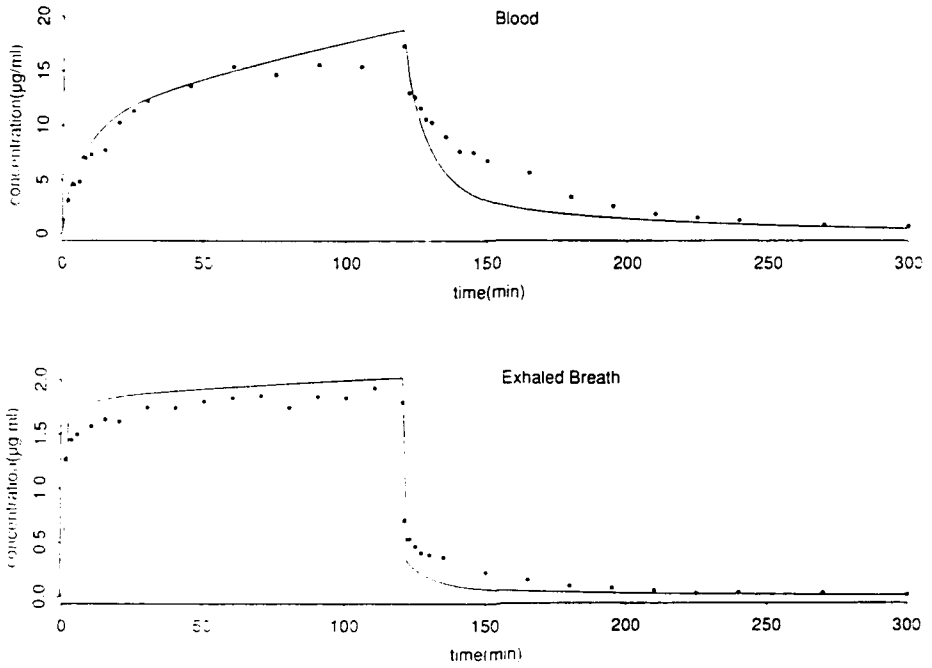

FIG. 3. Observed (●) and model-predicted (—) TCE concentrations in the arterial blood following a 2-hr, 500 ppm inhalation exposure. Each point represents the mean value for six rats.

first hour in the 500 ppm group. Predicted and observed postexposure blood concentrations compared favorably, however, for the 50 ppm rats.

Percentage systemic uptake of TCE was time- but not concentration-dependent (Fig. 4). Uptake exceeded 90% during the first 5 min in both exposure groups, but decreased rapidly over the next 30 min. Thereafter, there was a relatively slow decline in uptake for the remainder of the 2-hr exposure. Percentage uptake appeared to be somewhat higher in the 50 than the 500 ppm animals during much of the first hour, though at no time point was there a statistically significant difference. Percentage systemic uptake values ($\bar{x} \pm$ SE, $n = 6$) during the second hour of exposure for the 50 and 500 ppm groups were 69.9 ± 0.5% and 71.1 ± 0.8%, respectively. There was a total cumulative uptake ($\bar{x} \pm$ SE) during the 2-hr period of 2.96 ± 0.32 mg, or 8.4 mg/kg, in the 50 ppm animals and 24.3 ± 1.2 mg, or 73.3 mg/kg, in the 500 ppm animals (Fig. 5)

DISCUSSION

There is a lack of definitive information on the systemic uptake and disposition of inhaled TCE during exposures, largely due to technical difficulties in accurately monitoring TCE lev-

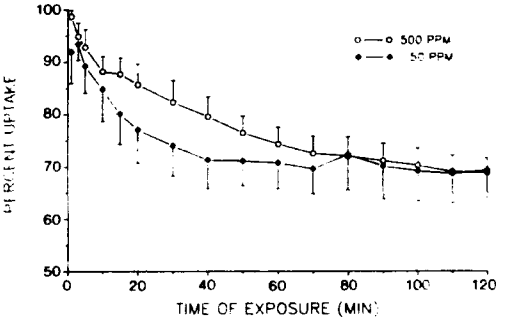

FIG. 4. Percentage systemic uptake of TCE over time during inhalation exposures. Rats inhaled 50 or 500 ppm TCE for 2 hr. Each point represents the mean ± SE for six rats. Percentage uptake of the inhaled dose was determined after 1, 3, 5, 10, 15, and 20 min and at 10-min intervals thereafter.

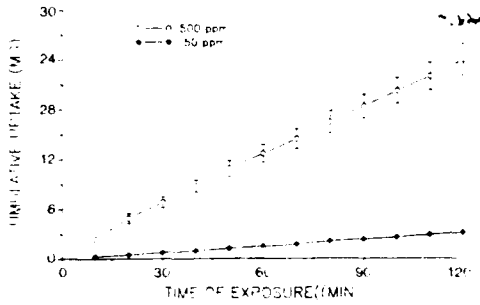

FIG. 5. Cumulative uptake of TCE during inhalation exposures. Rats inhaled 50 or 500 ppm TCE for 2 hr. The quantity of inhaled TCE retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled TCE concentrations. Each point represents the mean ± SE for six rats.

els in the blood and breath of laboratory animals and humans. It might be anticipated that TCE would behave similarly to other relatively water-insoluble gases. This proved to be the case in the current study. As TCE is a small, uncharged, lipophilic molecule, it is readily absorbed across membranes of the pulmonary capillary bed into the systemic circulation. The net rate of transfer from alveoli to blood is initially very rapid, but becomes progressively slower as the chemical accumulates in the blood and tissues. The approach to equilibrium in the blood and exhaled breath is quite rapid, indicative of TCE's relatively low solubility in blood and the slow perfusion of adipose tissue, the major site of deposition of the chemical. These processes are reflected by the time-course of systemic uptake of TCE, where percentage uptake decreases over time from ≥95% at the beginning of exposures to relatively constant levels of 69–71% at near steady-state. Studies in humans reveal lower uptake of inhaled TCE, with values ranging from 44 to 58% (Bartonicek, 1962; Astrand and Ovrum, 1976; Monster *et al.*, 1976, 1979). The greater percentage uptake in rats can be attributed in part to a difference in blood:air partition coefficients, in that values for the rat are 2½ to 3 times higher than for humans (Sato *et al.*, 1977; Gargas *et al.*, 1989). Other contributing factors to the species difference likely include the higher respiratory rate and cardiac output of the rat, as well as its greater apparent capacity to metabolize TCE.

Sequential measurements of TCE uptake during the 2-hr inhalation sessions made it possible to accurately monitor the cumulative uptake (i.e., quantity retained in the body, or absorbed dose) of the chemical. There was a cumulative uptake of 8.4 mg/kg in rats inhaling 50 ppm TCE for 2 hr. When adjusted for exposure concentration and duration, this value is about four times the cumulative uptake reported by Monster *et al.* (1979) in humans inhaling 70 ppm TCE for 4 hr. Thus, rats received a substantially greater systemic dose of TCE on a mg/kg bw basis than do humans at equivalent inhaled concentrations.

Findings in the current study indicate that the rat's capacity to assimilate and metabolize TCE is exceeded during the course of the 2-hr, 500 ppm exposure. Although exhaled breath levels of TCE were directly proportional to the inhaled concentration, arterial blood levels rose 25- to 30-fold with the 10-fold increase in exposure. The blood levels of the 500 ppm animals progressively increased over the 2-hr period, rather than approaching equilibrium as was the case at 50 ppm. Stott *et al.* (1982) saw evidence of metabolic saturation in rats exposed for 6 hr to 600 ppm [^{14}C]TCE. Metabolic saturation was manifest by a decrease in metabolism and increase in exhalation of TCE, when the exposure level was increased from 10 to 600 ppm. Filser and Bolt (1979) calculated the saturation point for TCE metabolism to be 65 ppm on the basis of indirect vapor uptake studies in male rats. Andersen *et al.* (1987), also utilizing data from gas uptake experiments in male rats, determined the V_{max} for TCE to be 11 mg/kg/hr. In the present study, the 10-fold increase in inhaled concentration (i.e., 50 to 500 ppm) resulted in an 8.7-fold increase in cumulative uptake, or total absorbed dose. Thus, metabolic saturation apparently commenced during the course of exposure, when there had

been systemic uptake of a finite quantity of TCE.

The capacity to metabolize TCE has been demonstrated by other investigators to be species-dependent. The metabolic saturation observed by Stott *et al.* (1982) in rats inhaling 600 ppm TCE for 6 hr was not seen at this exposure level in mice. The dose of TCE required to produce metabolic saturation in humans has not been clearly defined. Astrand and Ovrum (1976) saw no evidence of metabolic saturation in men inhaling 100 or 200 ppm TCE for up to 2 hr, in that percentage uptake was constant and absorbed dose was directly proportional to the inhaled concentration. Ikeda *et al.* (1972) reported that urinary concentrations of total trichloro compounds and trichloroethanol in occupationally exposed workers were proportional to inhaled concentrations of up to 175 ppm, but that there was a relative decrease in trichloroacetic acid at exposures above 50 ppm.

The major routes of elimination of TCE are metabolism and exhalation of the parent compound. The elimination of TCE in the exhaled breath generally paralleled elimination of the chemical from the bloodstream of rats in the present investigation. This pattern of elimination of TCE in the blood and breath is also typically seen in humans, although clearance is prolonged (Sato *et al.*, 1977; Nomiyama and Nomiyama, 1974). Despite species differences in TCE kinetics, comparable exhaled breath levels have been observed in rats and humans postexposure. Accounting for differences in exposure concentration, the postexposure exhaled breath levels of TCE from several studies in humans (Kimmerle and Eben, 1973a; Stewart *et al.*, 1974; Monster *et al.*, 1979) were similar in magnitude to the values of TCE eliminated in the exhaled breath of rats following inhalation exposure in the current investigation. Stewart *et al.* (1974), for example, measured concentrations of 0.70 and 0.28 ppm TCE in the exhaled breath of human subjects 30 and 120 min after termination of a 3-hr, 20 ppm inhalation exposure. Assuming a linear scaledown of the 50 ppm data from the current study, the TCE concentrations in the expired air of rats at these two time-points would be 0.92 and 0.28 ppm, respectively. Such a similarity in the magnitude of exhaled breath levels in humans and rats was also noted recently for 1,1,1-trichloroethane (Dallas *et al.*, 1989). The rat blood:air partition coefficient for each VOC is significantly higher than that for humans (Gargas *et al.*, 1989). Although this difference alone would result in greater respiratory elimination of the VOCs by humans, it is apparently offset by other factors, including the higher respiratory and circulatory rates of the rat.

PB-PK models for TCE have been developed by several groups of investigators. Sato *et al.* (1977) formulated a PB-PK model for respiratory exposure of humans to TCE. The model included three compartments, with intercompartment exchange of TCE governed solely by intertissue diffusion. Metabolic and respiratory excretion were assumed to occur in the richly perfused tissue compartment. Fernandez *et al.* (1977) constructed a more complete PB-PK model, which accurately predicted respiratory elimination of TCE and cumulative urinary excretion of TCE metabolites in humans. This model included the three compartments of Sato *et al.* (1977), as well as a liver compartment with blood-flow-limited metabolism and a lung compartment for respiratory absorption and elimination of TCE. Andersen *et al.* (1987) used a PB-PK model analogous to that of Ramsey and Andersen (1984) to predict the influence of competitive metabolic inhibition on uptake of inhaled TCE in rats. Fisher *et al.* (1989) subsequently modified the Ramsey and Andersen (1984) model to simulate the kinetics of TCE and trichloroacetic acid in the pregnant rat following inhalation and ingestion of TCE. Additional compartments (i.e., mammary tissue, placenta, and fetus) were incorporated into the model, and allowance was made for certain physiological changes which occur during pregnancy. The PB-PK model of Fisher *et al.* (1989) provided a good representation of TCE and trichloroacetic acid levels mea-

sured experimentally in maternal and fetal blood at a limited number of times postexposure. This model has been extended recently to predict the kinetics of TCE and trichloroacetic acid in lactating rats and nursing pups (Fisher *et al.*, 1990).

The PB-PK model used in the current investigation accurately predicted the time-courses of TCE concentrations in the blood and exhaled breath of rats during and following inhalation exposure to 50 and 500 ppm TCE. The model is similar to those of Ramsey and Andersen (1984) and Angelo and Pritchard (1984). Our PB-PK model differs in that it includes a separate lung tissue compartment and a lung:alveolar mass transfer coefficient, which describes the bidirectional transfer of TCE across the alveolar membrane. It is only necessary to alter the experimentally determined inhaled concentration and minute volume in order to obtain simulations of TCE kinetics under different inhalation exposure scenarios. Metabolic saturation, manifest by the progressive, disproportionate increase in blood levels in the high-dose (i.e., 500 ppm) animals, was accurately forecast. There was also good agreement between predicted and observed blood and breath levels during most of the postexposure period. Previous investigators, including Fisher *et al.* (1989), have had the use of very limited experimental data sets for assessing the precision of their model predictions.

Health risk assessments of VOCs such as TCE require a careful selection of the measure of dose. Areas under blood and tissue concentration versus time curves have been advocated as logical measures of target organ dose (Andersen, 1987). The most appropriate chemical species to measure for TCE depends upon which toxic effect is of interest. TCE appears to be primarily responsible for CNS depression and cardiac arrhythmias. As it is unclear which metabolite(s) should be used as dose measures, or surrogates for TCE-induced cytotoxicity and mutagenicity/carcinogenicity, the amount of reactive intermediate (i.e., toxicologically effective dose) has been equated to the total amount of TCE metabolized by the liver (Bruckner *et al.*, 1989). The NRC (1986) applied the PB-PK model of Ramsey and Andersen (1984) to calculate the toxicologically effective dose formed in the liver of rats during TCE inhalation exposure. Bogen (1988) has more recently applied the model of Ramsey and Andersen (1984) to predict relationships between the administered dose and the toxicologically active, or metabolized dose of TCE in humans. Comprehensive TCE time-course data sets, however, have not been available for rigorous validation of model predictions. The next logical step in this direction will be to obtain TCE/metabolite tissue concentration versus time data sets through direct measurement studies.

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ANALYSES OF VOLATILE C2 HALOETHANES AND HALOETHENES IN TISSUES: SAMPLE PREPARATION AND EXTRACTION

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ABSTRACT

Characterization of the systemic uptake, distribution and elimination of volatile organic compounds (VOCs) requires reliable analytical techniques for measuring the concentration of the chemicals in different tissues of the body. An extraction procedure was developed which minimized loss of the readily volatilizable compounds, so that they could subsequently be quantified by headspace gas chromatography. The procedure was evaluated using four C2 halocarbons [i.e., perchloroethylene (PER), 1,1,1-trichloroethane (TRI), 1,1,2,2-tetrachloroethane (TET), and 1,1,2-trichloroethylene (TCE)] of varying physicochemical properties. Portions of 0.5 to 1 g of liver, kidney, brain, heart, lung, skeletal muscle, fat and blood from rats were spiked with PER to yield a theoretical concentration of 4 μg/g tissue. Two homogenization procedures were evaluated: (a) tissues were homogenized in saline, followed by isooctane extraction; and (b) tissues were homogenized in isooctane and saline (4:1, v:v). The latter approach resulted in a significantly higher percent recovery of PER from most tissues. Neither homogenization nor the presence of saline affected PER standards prepared in isooctane. It was observed that the volume of the aliquot of isooctane taken for PER analysis was important, in that aliquots >25μl could not be used. PER concentrations were determined in tissues of rats following *in vivo* (i.e., intraarterial administration) of the halocarbon using the latter (i.e., isooctane) homogenization approach. This approach was also employed to examine the efficiency of recovery of PER, TET, TRI and TCE from seven tissues and from blood. Percent recoveries of each of the four halocarbons ranged from 73-104% for the seven spiked tissues. The recoveries did not appear to be tissue-dependent, despite differences in homogenization time required for different tissues. Recovery, however, did vary somewhat with test chemical, with the least volatile, most lipophilic compounds exhibiting the highest recovery.

INTRODUCTION

Short-chain aliphatic halogenated hydrocarbons (halocarbons) are a class of volatile organic compounds (VOCs) of increasing concern, due to their widespread occurrence as environmental contaminants, and the potential risks they pose to health. Exposure to halocarbons can result in toxic injury of a number of organ systems in animals and humans. Central nervous system (CNS) dysfunction results from overexposure to most halocarbons and other VOCs [1,2]. CNS depressant effects have been directly correlated with the concentration of hydrocarbons in the brain [3,4]. Significant liver and kidney damage can be caused by certain halocarbons [5-7], while some members of this chemical class are carcinogenic in different organ systems in animals [8-10].

Pharmacokinetic studies of halocarbons are needed in order to elucidate target organ uptake, deposition, and elimination of the chemicals. The magnitude of toxic effect in an organ is, of course, dependent upon the amount of chemical present in the tissue. Pharmacokinetic studies conducted to date have primarily involved measurement of concentrations of halocarbons in blood and exhaled breath [11-15]. The limited (i.e., at a single time-point) tissue measurements conducted in some of these studies employed ^{14}C-labeled halocarbons. Measurement of total radioactivity does not delineate between the parent compound, metabolites, and ^{14}C which has entered the body's carbon pool. There have been a limited number of investigations, in which time-courses of tissue deposition of inhaled hydrocarbons have been delineated [3,4,16-18]. In these studies, the tissues were extracted with a solvent and the parent compounds quantified by gas chromatography (GC) or GC/mass spectral analysis. No reports of the time-course of uptake and elimination of halocarbons in tissues were found in the literature.

A variety of approaches have been used for analysis of VOCs in blood and tissues. One technique is to simply inject blood and tissue homogenates directly into a GC [16,19-21]. Major drawbacks of direct injection of biological materials are that the materials cause matrix interferences and that contamination markedly shortens the GC column's lifetime. Solvent extraction is a widely used approach for measuring concentrations of VOCs in blood and tissues. An aliquot of the solvent may be directly injected into the GC column [17,18,22,23]. Since this method typically involves a one-step extraction of the VOC with the solvent, limited sensitivity and interference by other lipophilic compounds can be problematic. In order to circumvent these difficulties, more complex procedures have been employed. One entails evaporation of the solvent extract and trapping of the VOC analyate on a Tenax® column [24]. Another involves heating biological samples within a purging device [25-29], with subsequent retention of the analyte on an adsorbant such as Tenax®. Such approaches are technically difficult and time consuming. Headspace analysis has proven to be a sensitive and more efficient means of measuring VOC concentrations in blood samples [14,15,30,31]. No one, however, appears to have reported a suitable technique for quantification of halocarbons or other VOCs in tissues.

In light of the foregoing, it is apparent that there is a need for a rapid, sensitive analytical procedure for reliably measuring the concentrations of halocarbons and other VOCs in different tissues. The overall objective of this project was to adapt the headspace technique previously used for analysis of blood samples for measurement of C2 halocarbons in different tissues. A major focus of the work was development of a procedure for conservation of the analylate (i.e., minimization of loss by volatilization) during preparation and extraction of the tissue samples. Two C2 haloalkananes and two C2 haloalkenes

were employed, in order to assess the utility of the procedure for extraction and subsequent analyses of VOCs with different physicochemical properties.

MATERIALS AND METHODS

Apparatus

1. Gas chromatograph. Model 300 (Perkin-Elmer Co. Norwalk, CT), HS-6 autosampler, Model 5890 equipped with an electron capture detector and a 19395A autosampler (Hewlett-Packard Co. Avondale, PA)
2. Stainless-steel column. 6' x 1/8", 10% FFAP (Alltech Associates, Deerfield, IL), 3% SP 1000 (Supelco, Bellefonte, PA), or 3% OV-17 (Alltech Associates, Deerfield, IL)
3. IEC Centra-7r Refrigerated Centrifuge (International Equipment Company, Needham, MA)
4. Homogenizer, Janke & Kunkel, Ika-Werk, Ultra-Turrax SDT (Tekmar Company, Cincinnati, OH)
5. Rubber septa (PTFE Coated Butyl Rubber), 8-ml glass vials, aluminum caps (Perkin-Elmer Co., Norwalk, CT)

Test Chemicals

1. 1,1,2,2-Tetrachloroethylene, Perchloroethylene (PER). 99% purity (Aldrich Chemical Company Inc. Milwaukee, WI)
2. 1,1,2,2-Tetrachloroethane (TET). 97% purity (Aldrich Company Inc. Milwaukee, WI)
3. 1,1,1-Trichloroethane (TRI). 99% purity (J.T. Baker Chemical Co. Phillipsburg, NJ)

4. 1,1,2-Trichloroethylene (TCE). 99% purity (J.T. Baker Chemical Co. Phillipsburg, NJ)

5. Isooctane. 99.98% purity (J.T. Baker Chemical Co., Phillipsburg, NJ)

6. Ether, Anhydrous. (J.T. Baker Chemical Co. Phillipsburg, NJ)

Tissue Homogenization and Extraction Procedures

Twelve-week-old male Sprague-Dawley rats were obtained from Charles River Laboratories (Raleigh, NC). After 2 to 3 weeks, groups of 4 or 8 animals (bw = 325-375 g) were anesthetized with ether. One-ml blood samples were withdrawn by closed chest cardiac puncture. From 0.5- to 1-g portions of liver, kidney, brain, heart, lung, perirenal fat, and skeletal muscle were removed and placed onto ice. Each tissue was spiked with PER, TET, TCE or TRI, by injection of 4 μl/g tissue of a solution containing 1 mg halocarbon/ml isooctane. Two homogenization approaches were evaluated using PER. In the first, the tissues were immediately transferred after halocarbon injection to previously chilled 20-ml glass vials containing 4 ml of ice-cold saline. The tissues were allowed to remain in the tightly capped vials for approximately 30 min, before being homogenized for an established time interval with a Tekmar tissue homogenizer. These times were kept as brief as possible in order to minimize volatilization of the halocarbons. Brain, liver, and fat were the most easily homogenized, requiring only 3-4 sec. Kidney, lung and heart required 5-8 sec. Skeletal muscle was the most difficult to homogenize, in that it required 20 sec. Eight ml of isooctane were added to the homogenates, which were then vortexed for 30 sec and centrifuged at 1800 x g for 10 min at 4°C in capped vials. An aliquot of the isooctane layer was transferred to capped vials for headspace analysis. In the second approach, tissues were immediately transferred after halocarbon

injection to previously chilled 20-ml glass vials containing 2 ml of ice-cold saline and 8 ml of isooctane. Each tissue was then homogenized, vortexed and centrifuged as before. An aliquot of the isooctane layer was taken for headspace analysis. Only the latter (i.e., isooctane homogenization) approach was subsequently used for determination of TCE, TRI, TET and PER in tissues, except in the aliquot volume study.

Isooctane Aliquot Volume Study

An experiment was conducted to determine the effect of aliquot volume on the linearity of halocarbon quantification. As in other *in vitro* experiments, a Hamilton gas-tight syringe was used to inject the chemical into the center of the tissue cubes. Four μl of PER were injected into samples of blood and each of the seven tissues. The tissues were homogenized in saline and subsequently extracted with isooctane as described previously. From 5- to 100-μl aliquots of isooctane extract were withdrawn with a pipet and transferred to 8-ml headspace vials. These vials were capped immediately with rubber septa and a spring washer and crimped to insure an airtight seal. Each sample vial was then placed into the autosampler unit of the gas chromatograph.

Headspace Gas Chromatographic (GC) Techniques

For all the experiments with PER, the GC operating conditions were: headspace sampler temperature, 90°C; injection port temperature, 200°C; column temperature, 110°C; detector temperature, 400°C; column packing, 10% FFAP; flow rate for argon/methane carrier gas, 60 ml/min. Operating conditions for TET were: headspace sampler temperature, 100°C; injection port temperature, 200°C; column temperature, 150°C; detector temperature, 400°C; column packing, 3% OV-17;

flow rate for argon/methane carrier gas, 60 ml/min. Operating conditions for TRI and TCE were: headspace sampler temperature, 55°C; injection port temperature 150°C; column temperature, 60°C; detector temperature, 400°C; column packing, 3% SP 1000. Except for the isooctane aliquot volume study, all analyses were conducted using a 20-μl aliquot of isooctane in the 8-ml headspace vials. Each sample vial was heated thermostatically for 10 min in the GC autosampler unit, pressurized for 30 sec with the carrier gas, and injected automatically by a solenoid-controlled mechanism into the GC column.

The aforementioned conditions resulted in vaporization of the halocarbons in the sample vials, since each VOC was heated to a temperature slightly below its boiling point, and the vial subsequently pressurized and vented into the GC. An experiment was conducted to assess the influence of heating time on quantification of PER. Heating sample vials 5 min or longer resulted in a constant GC detector response (i.e., area under the curve) for a series of known quantities of PER (data not shown).

As saline was utilized in the tissue homogenization procedures, an experiment was conducted to determine whether the presence of saline influenced standard curves. PER was incorporated into four different solvent systems: 8 ml isooctane; 2 ml saline + 8 ml isooctane; 4 ml saline + 8 ml isooctane, and 4 ml saline + 8 ml isooctane homogenized for 30 sec. Each solution was vortexed for 30 sec. Aliquots of 1 to 25 μl of the isooctane layer, equivalent to 1 to 25 ng PER, were subjected to GC headspace analysis. Standard curves were generated on the basis of the GC peak area plots. The slopes, intercepts and correlation coefficients of the curves were compared. As described in the Results section, the standard curves did not vary significantly from one solvent system to another. Therefore, the simplest system was subsequently employed for

preparation of standard solutions of PER, TET, TCE and TRI (i.e., an appropriate amount of halocarbon was dissolved in isooctane alone). Standard curves for each compound were generated the same day that sample analyses were performed, using the same analytical conditions.

The limit of detection of the GC assay was determined by the method described by MacDougall et al. [32]. A signal: noise ratio of 3 or greater was considered as the limit of detection. The presence of background noise and any interfering peaks was assessed in air and in isooctane samples. Neither was observed, as the detector baseline was consistently stable. The limit of detection for TRI, TCE, PER and TET was found to be 1 ng. This amount is equivalent to 8.4, 8.5, 6.7 and 6.6 parts of chemical per billion parts of air for TRI, TCE, PER and TET, respectively.

In vivo Tissue Measurements

The concentration of PER was determined in tissues of rats following intraarterial administration of the compound. Male Sprague-Dawley rats of 325-375 g from Charles River Laboratories (Raleigh, NC) were surgically implanted with an indwelling carotid artery cannula. The cannula exited the body behind the head, so the animal could not disturb the cannula, but have freedom of movement. Food was withheld during an 18-hr recovery period before PER administration. PER was incorporated into undiluted polyethylene glycol 400, and a dose of 10 mg PER/kg bw injected as a single bolus into the arterial cannula. Each animal was anesthetized with ether 1 hr after dosing, and blood taken by closed-chest cardiac puncture. One-g portions of liver, kidney, brain, heart, lung, perirenal fat and skeletal muscle were excised within 2.5- to 3-min from each animal and immediately placed into chilled vials containing 2 ml saline and

8 ml isooctane. The samples were processed for PER analysis using the isooctane homogenization procedure described previously.

Statistics

Comparisons of the percent recovery of PER from tissues and blood, using the two homogenization procedures, were made using Student's t test. A two-way analysis of variance was utilized to assess the significance of variances among standard curves for the different saline-isooctane mixtures. Values were considered significantly different at $p < 0.05$.

RESULTS

Results of the study on the effect of isooctane aliquot volume on the linearity of halocarbon quantification are presented in Fig. 1. A very similar pattern was observed for all tissues studied. The quantity of PER increased linearally with increasing aliquot volume up to 25 μl. Use of larger aliquots of isooctane (i.e., 50 and 100 μl) did not result in any further increase in the amount of measurable PER.

Percent recovery values obtained using the saline and isooctane homogenization approaches for PER are contrasted in Table 1. Recoveries were quite good with both procedures, in that values ranged from approximately 72 to 104%. Percent recoveries of PER from kidney, fat, lung, muscle, and brain were significantly higher when the tissues were homogenized directly in isooctane. Recovery of PER from liver, heart, and blood did not differ significantly for the two procedures.

Percent recoveries of PER, TET, TCE and TRI from spiked tissues, utilizing the isooctane homogenization procedure, are tabulated in Table 2. Recovery of

TET was generally higher than was the case for the other three chemicals. Percent recovery of TCE was generally the lowest of the four chemicals, with no mean values exceeding 88% for any tissue. Indeed, the lowest recovery of TET (i.e., from fat) was greater than the highest recovery of TCE (i.e., from muscle). The mean percent recoveries of PER, TET, and TRI from fat were quite similar (within 2%). TCE recovery (73%) from fat was the lowest for any chemical from any tissue. The range in values for the different tissues was the smallest for TRI (i.e., less than 6%), and the largest for TCE (i.e., 14.9%). Percent recovery of the four volatile chemicals from tissues with the shortest homogenization time (i.e., liver, fat, and brain) was not substantially different from other tissues. Unexpectedly, there was relatively high recovery of all four halocarbons from skeletal muscle, the tissue requiring the longest homogenization time. No tissue consistently exhibited higher or lower recover values for any of the four chemicals.

Standard curves for PER standards, prepared using four different solvent and saline mixtures, are shown in Fig. 2. The linear regression equations were determined to be $y = 25.9x - 13.3$ for 8 ml isooctane, $25.9x - 8.4$ for the 2 ml saline/8 ml isooctane mixture, $25.8x - 8.0$ for the 4 ml saline/8 ml nonhomogenized isooctane mixture, and $25.2x - 3.6$ for the 4 ml saline/8 ml homogenized isooctane mixture. For all of the data considered together, the linear regression equation was $y = 24.3x - 6.9$. Thus, there was no statistically significant difference between the regression equations for the four solvent systems. Therefore, neither the presence of saline nor homogenization significantly affected standard curves for PER.

Concentrations of PER, measured in tissues of rats 1 hr following intraarterial administration of a single 10 mg/kg dose of PER, are shown in Table

3. PER levels in the fat were an order of magnitude or more higher than in any other tissue sampled. The concentrations of PER measured in the liver, kidney, heart, and lung were relatively consistent (i.e., within 25% of each other). Brain levels of PER were approximately 2-fold higher than in these organs, while the blood and skeletal muscle exhibited the lowest concentrations.

DISCUSSION

Gas chromatographic (GC) techniques are routinely used to determine levels of VOCs in environmental and biological samples. Wallace and his colleagues [33] utilized GC purge and trap techniques to conduct large-scale surveys of human exposure to VOCs in drinking water, indoor and outdoor air. GC purge and trap techniques have also been used successfully to measure concentrations of VOCs in human blood [27,29,30,34] milk [26,27] and urine [27]. These assays are precise and quite sensitive, as many of the investigators employed GC-mass spectrometric computer analyses. Other investigators have used static GC headspace methods to quantify halocarbons and other VOCs in blood [14,15,30,31]. Such headspace analyses offer the advantages of speed and simplicity, such that large numbers of samples can be assayed daily using a GC equipped with an autosampler. Although each of the aforementioned techniques generally work well for air and liquids, little attention has been devoted to adapting them for measurement of VOCs in solid tissues.

A practical technique for processing and extracting C2 halocarbons from tissues for subsequent GC headspace analyses is reported here. Several approaches for determination of VOCs in tissues have been employed previously, with limited success. Direct injections of tissue homogenates or solvent extracts of homogenates have a number of inherent problems, including loss of the

VOC by volatilization, GC column contamination, interference by biological matrices and lipophilic macromolecules, and limited sensitivity. One method for measuring toluene in blood and tissues involved extraction with methanol, selective adsorption onto Tenax®, and desorption from the Tenax® with heat into a GC [24]. A significant problem in measuring VOCs in solid tissues is volatization of the analyte during tissue processing. Peterson and Bruckner [24] attempted to overcome this difficulty by crushing the tissues with a rod under methanol within a closed container. This technique was reasonably successful (e.g., 73 and 92% recovery of toluene from liver and brain, respectively), but recoveries from other tissues were limited by incomplete maceration and escape of toluene from the maceration/extraction container. This technique was also labor intensive and time consuming, as was a purge and trap method described by Lin et al. [28] for measuring 1,1- and 1,2-dichloroethylene (1,2-DCE). The procedure of Lin et al. [28] involved thermal desorption of halocarbons from previously minced tissues within a purging device. The chemicals were subsequently trapped on a Tenax® column, and desorbed with heat into a GC. Mean recovery values for 1,2-DCE from liver, kidney, brain and adipose tissue were 60, 53, 63 and 93%, respectively [28]. The purge and trap technique of Pellizzari and co-workers [27,35] also resulted in low recovery (i.e., 13-80%) of a series of halocarbons from adipose tissue. The method involved transfer of 5-g portions of frozen adipose tissue to a 100-ml round-bottom purging flask maintained in an ice bath, addition of the halocarbon dissolved in distilled water, and maceration with a Virtis tissue homogenizer. The flask's contents were then heated to 50°C, stirred and purged with helium for 30 min, in order to transfer the analylate to a Tenax® column. These researchers [27,35] attributed their low recovery values

and marked intersample variability to halocarbon losses during tissue maceration and transfer, as well as retention of the analylate by complex matrices and lipophilic compounds. In contrast, the technique presented in the current paper is quite efficient, in that it involves a homogenization/extraction step and the relative ease and speed of GC headspace analysis. The method was also sensitive (i.e., limit of detection = 1 ng) and efficient, in that recoveries of four different halocarbons from a variety of tissues were quite high (i.e., 73-104%) and consistent (i.e., highest S.E. = 4.9%).

An important factor in the present procedure was the limitation in the volume of aliquot that could be employed in the headspace vials. As standard curve measurements were no longer linear at volumes above 25 μl, a 20-μl aliquot was selected for subsequent use. There was a statistically higher percent recovery from most tissues when using isooctane homogenization than when using saline homogenization. It appears that homogenization of tissues in an aqueous solution (i.e., saline), with subsequent extraction into isooctane, provided more opportunity for loss of the volatile chemicals through evaporation than did the single step of homogenization in isooctane. Isooctane proved to be superior to a variety of other organic solvents for extraction of all four halocarbons (unpublished data). Other solvents that were employed included methanol, ethylacetate, n-hexane, cyclohexane and toluene. Some solvents (e.g., ethylacetate) worked well for one halocarbon, but not for others. Isooctane provided the highest recovery without interfering peaks for all four halocarbons.

The applicability of this approach for analysis of C2 halocarbons was demonstrated by its use with two haloalkanes and two haloalkenes with differing physicochemical properties. As would be expected, the relative volatility of the chemicals affected percent recovery. The boiling points of TRI, TCE, PER, and

TET are 74, 86.7, 121, and 140.7°C, respectively [36]. TET, the least volatile chemical, exhibited the highest percent recovery from most tissues. Recovery of PER was also relatively high from each tissue except the heart. It is noteworthy that PER is the most lipophilic of the halocarbons [37], and therefore should be most efficiently extracted by isooctane. TCE generally exhibited the lowest recovery values, as would be anticipated from its relatively high volatility and low liphophilicity. Recovery of TRI was unexpectedly high from most tissues. One would predict that TRI recovery should also be relatively low, since it was the most volatile and one of the least lipophilic of the four compounds studied.

A basic tenet of toxicology is that of the dose-response relationship (i.e., the magnitude of toxic effect is a function of the administered dose). The concept of dose is now being refined, as it is recognized that the amount of chemically absorbed systemically (i.e., the internal dose) can vary significantly with route of exposure, dosing vehicle and animal species. The blood level over time following exposure has been accepted historically as an index of internal dose, but it often may not accurately reflect concentration of chemicals at sites of action within tissues. Thus, the most logical and precise measures of dose are time integrals of target organ concentrations of bioactive chemicals [38]. Unfortunately, there are a paucity of tissue level versus time data sets for VOCs, due largely to the technical difficulties and the inordinate time involved in quantification of these highly volatile compounds in individual samples. In order to derive appropriate time-course data, tissue concentrations must be measured sequentially during and post exposure in separate groups of animals, necessitating analysis of a large number of samples. A technique is presented here, which allows rapid processing and extraction of C2 halocarbons from a variety of organs, for subsequent GC headspace analyses. By use of such a

procedure, it should be possible to generate comprehensive tissue dose-time course data to correlate with toxicity data. Recognition and utilization of such information can substantially reduce uncertainties inherent in toxicity and carcinogenicity risk assessments of halocarbons, and other VOCs.

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Table 1

Effect of Homogenization Procedure on Percent Recovery of PER

	Saline Homogenization	Isooctane Homogenization
Liver	95.5 ± 9.9	89.6 ± 3.1
Kidney	69.0 ± 4.9***	86.7 ± 1.4***
Fat	73.8 ± 4.9*	88.2 ± 2.7*
Heart	75.8 ± 10.3	81.2 ± 1.2
Lung	78.8 ± 7.1**	99.1 ± 2.3**
Muscle	80.3 ± 6.3*	98.5 ± 2.9*
Brain	72.3 ± 5.7**	88.6 ± 2.0**
Blood	104.8 ± 5.5	95.4 ± 4.1

Each value represents the mean ± SE for recovery of 1,1,2,2-tetrachloroethylene (PER) from tissues of 8 rats for isooctane homogenization and 4 rats for saline homogenization.

* significant difference between procedures at $p < 0.05$.
** significant difference between procedures at $p < 0.01$.
*** significant difference between procedures at $p < 0.001$.

Table 2

Percent Recovery of C_2 Haloalkanes and Haloalkenes from Blood and Tissues

	PER	TET	TCE	TRI
Liver	89.6 ± 3.1	96.1 ± 2.1	86.0 ± 1.3	91.3 ± 4.0
Kidney	86.7 ± 1.4	97.9 ± 1.5	86.9 ± 1.6	88.4 ± 4.9
Fat	88.2 ± 2.7	89.9 ± 1.4	73.0 ± 1.5	88.7 ± 2.2
Heart	81.2 ± 1.2	98.1 ± 1.4	85.9 ± 3.5	89.6 ± 2.4
Lung	99.1 ± 2.3	96.1 ± 0.8	80.0 ± 0.7	89.7 ± 1.9
Muscle	98.5 ± 2.9	97.4 ± 1.1	87.9 ± 1.7	87.6 ± 4.9
Brain	88.6 ± 2.0	100.3 ± 3.1	80.7 ± 2.8	87.6 ± 4.6
Blood	95.4 ± 4.1	97.3 ± 2.1	85.9 ± 2.0	85.5 ± 3.7

Values represent the mean ± SE for measurement in spiked tissues taken from 8 rats.
Each spiked tissue was homogenized in 8 ml ice-cold isooctane and 2 ml saline, vortexed, centrifuged at 4°C, and an aliquot of the isooctane assayed by headspace GC as described in the Materials and Methods.

Table 3

Tissue Concentrations of PER in Rats Following *In Vivo* Exposure

Tissue	PER Concentration* (μg/g)
Liver	2.4 ± 0.5
Kidney	2.7 ± 0.4
Fat	48.1 ± 4.1
Heart	3.0 ± 0.2
Lung	2.3 ± 0.2
Muscle	1.9 ± 0.2
Brain	4.7 ± 0.4
Blood	1.3 ± 0.1

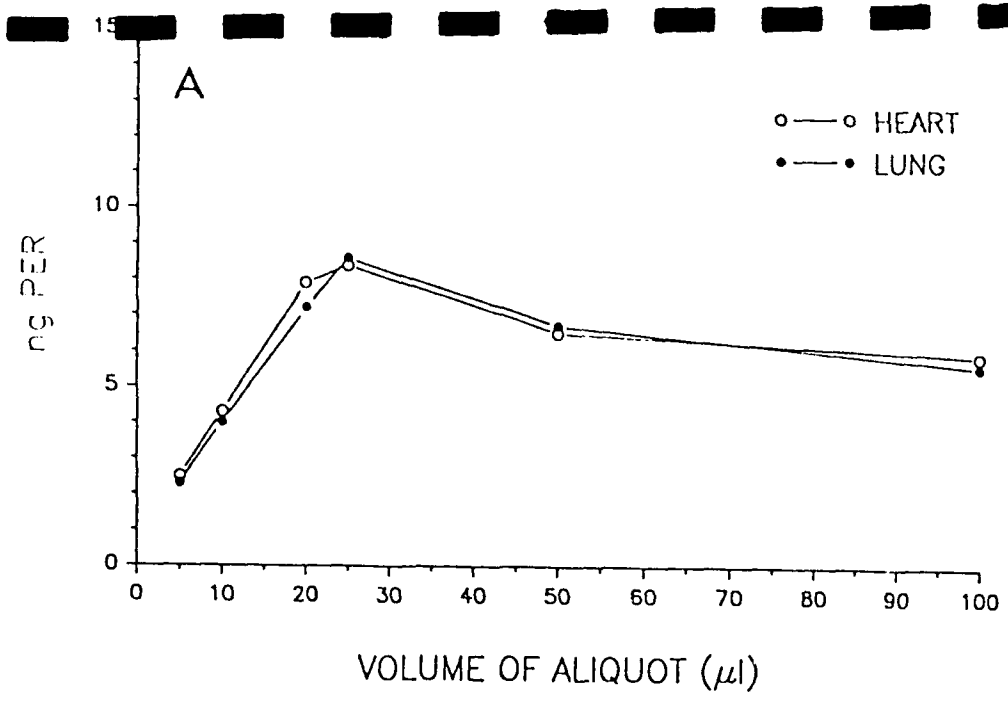
Each animal was sacrificed 1 hr after intraarterial administration of 10 mg 1,1,2,2-tetrachloroethylene (PER)/kg bw.

*Values represent the mean ± SE for 4 rats.

FIGURE LEGENDS

Fig. 1 Effect of isooctane aliquot volume on linearity of PER content in samples from heart and lung (A), fat and blood (B), brain and muscle (C), and liver and kidney (D). Aliquot volumes of 5, 10, 20, 25, 50 and 100 μl were withdrawn from the organic phase of the tissue homogenate and analyzed by GC headspace analysis.

Fig. 2 Standard curves for PER using four solvent and saline combinations and homogenization: 8 ml isooctane (ISO); 8 ml isooctane + 2 ml saline (ISO + 2ML SAL); 8 ml isooctane + 4 ml saline (ISO + 4 ML SAL) and 8 ml isooctane + 4 ml saline, homogenized for 30 sec. The detector response, presented here as area under the curve (AUC), is plotted against amount of PER. The regression equation for the data presented here is $y = 24.3 - 6.9$.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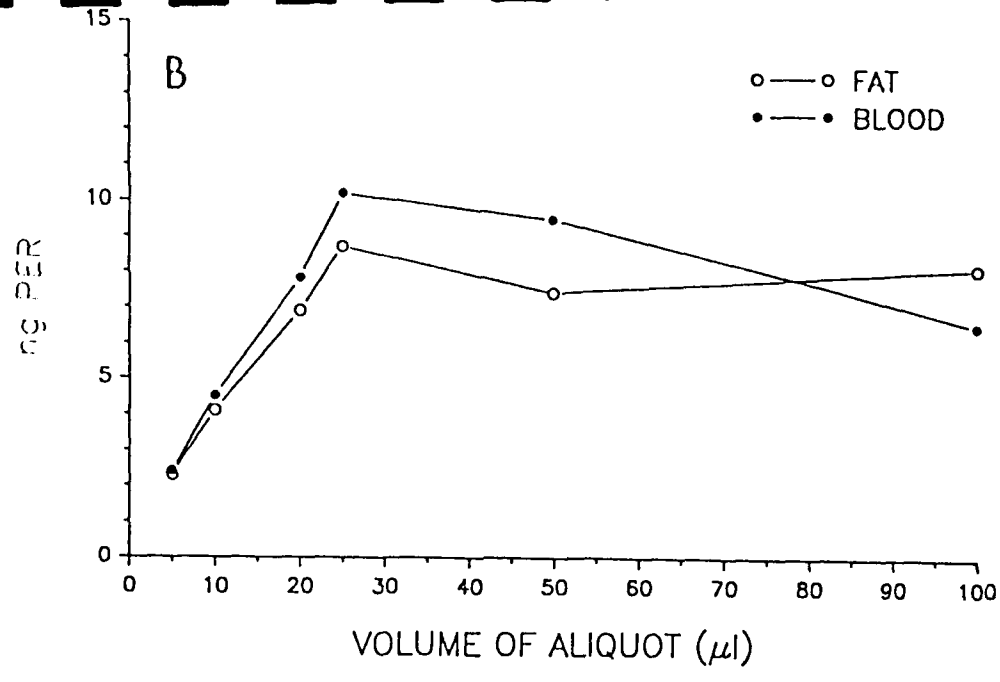
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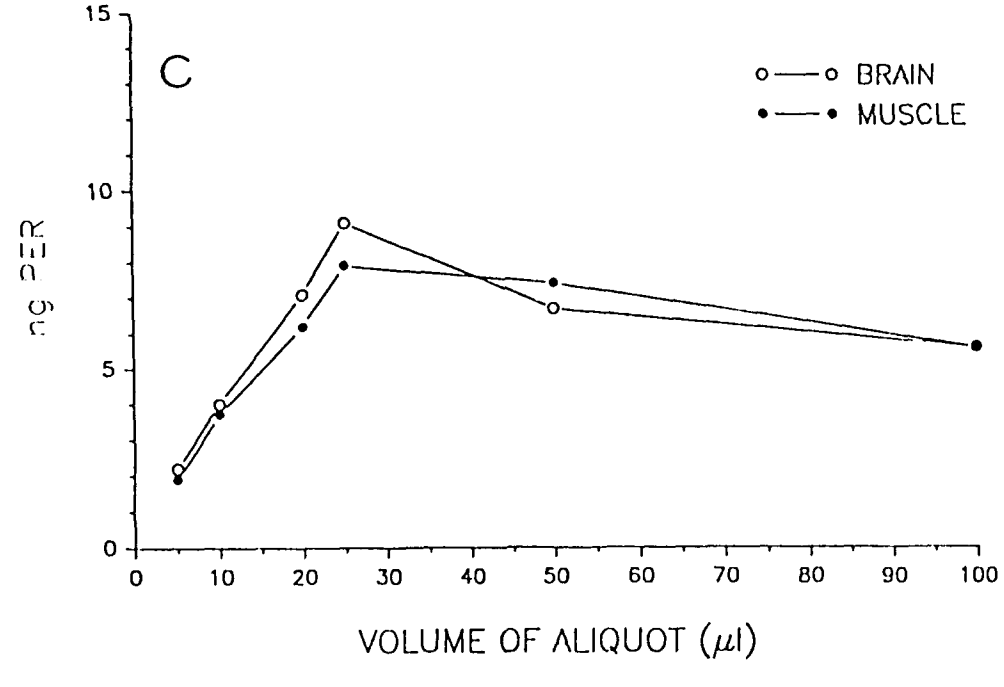
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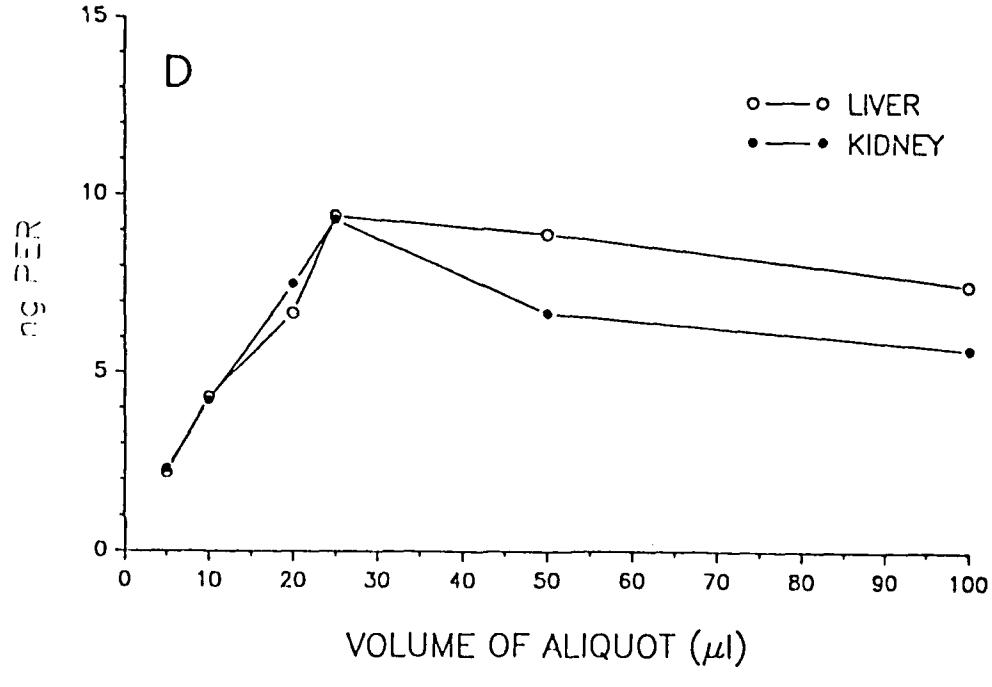
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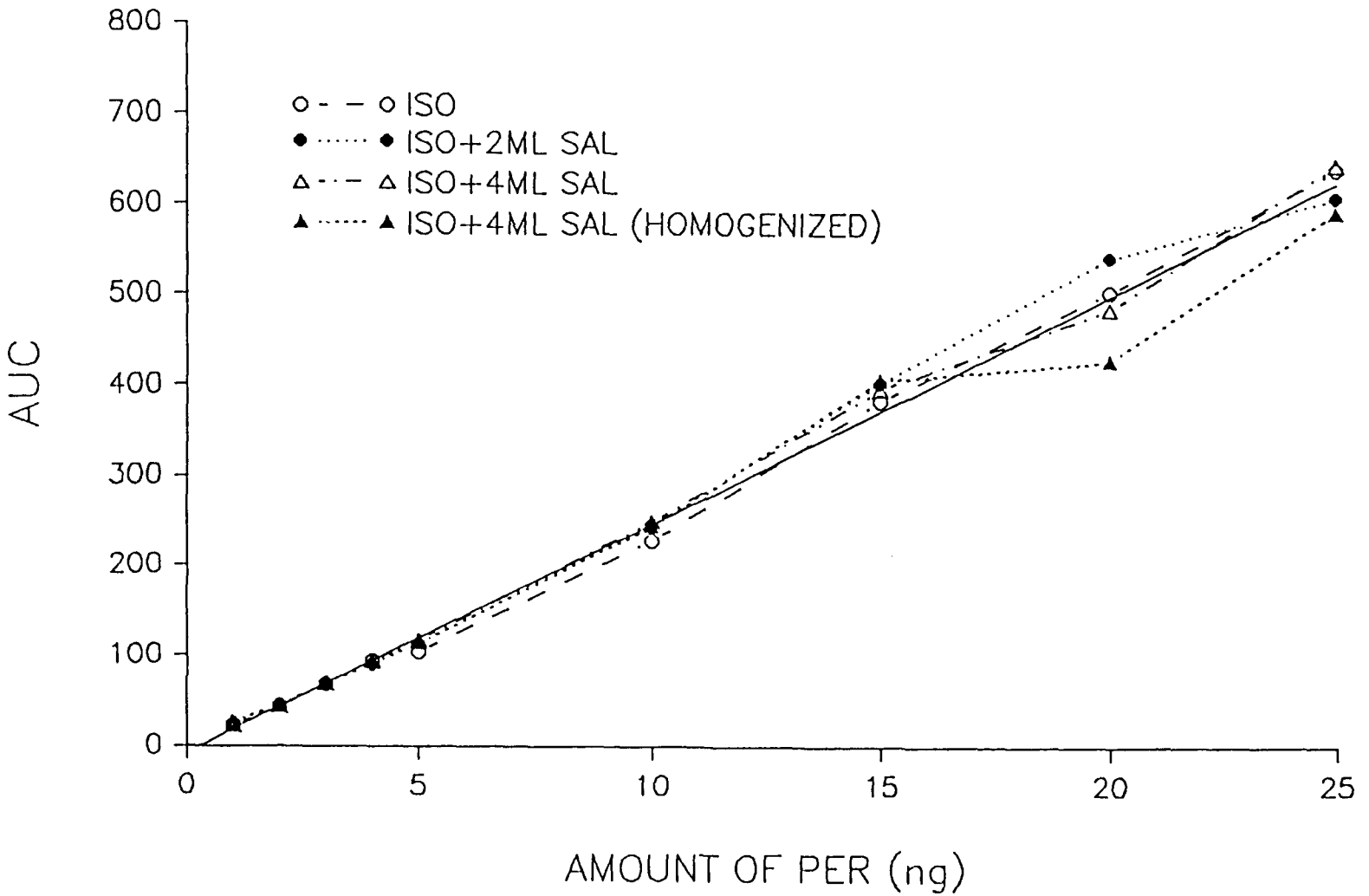
800
700
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400
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ISO
ISO+2ML SAL
ISO+4ML SAL
ISO+4ML SAL (HOMOGENIZED)
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25
AMOUNT OF PER (ng)

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Development of a Physiologically Based Pharmacokinetic Model for Inhaled Perchloroethylene in Rats

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ABSTRACT

The pharmacokinetics of perchloroethylene (PCE) was studied in male Sprague-Dawley rats to characterize and quantify systemic uptake and respiratory elimination by direct measurements of the inhaled compound. Fifty or 500 ppm per was inhaled for 2 hr through a one-way breathing valve by unanesthetized rats of 325-375 g. Repetitive samples of the separate inhaled and exhaled breath streams, as well as arterial blood, were collected during and following PCE inhalation and analyzed by gas chromatography. PCE exhaled breath and alveolar levels increased rapidly after the initiation of exposure to near steady-state within about 60 min. They were then directly proportional to the exposure concentration. Uptake of PCE in the blood was also rapid, but blood levels continued to increase progressively over the course of the 2-hr exposure at both dose levels. Cumulative uptake, or total absorbed dose, was proportional to the inhalation exposure level. A blood flow limited physiologically-based model was characterized with PCE eliminated in the exhaled breath and to a limited extent by liver metabolism. PCE concentrations in the blood and exhaled breath were well predicted by the PBPK model. The usefulness of model simulations in predicting systemically absorbed doses of PCE was demonstrated, which can have utility in risk assessments involving the internal dose of volatile organics.

INTRODUCTION

Perchloroethylene (1,1,2,2-tetrachloroethylene, PCE) is a volatile organic compound (VOC) which is used in large quantities in industry for metal degreasing, dry cleaning fabrics and textiles, and as an intermediate for the production of other chemicals. Approximately 500,000 workers in the United States are estimated to be at risk of occupational exposure to PCE (NIOSH, 1978). Measurements of workplace air concentrations of PCE in the dry cleaning industry have determined mean time-weighted-averages (TWA, 8 hr), from 28.2 to 88.2 ppm (Materna, 1985), 4.0-149 ppm (Ludwig et al., 1983), and as high as 178 ppm (HSDB, 1987). Elevated concentrations of PER in indoor air have also been reported for residences with PER-contaminated water supplies (Highland et al., 1985; Andelman, 1985). Central nervous system (CNS) effects such as dizziness, headache, sleepiness, and incoordination have been reported in humans from acute PCE exposures of 100 to 200 ppm and above (Hake and Stewart, 1977; Stewart et al., 1970). Acute inhalation exposures to PCE in animals have also been reported to result in mild hepatotoxicity (Kylin et al., 1963), biochemical changes in the brain such as reduced RNA content (Savolainen et al., 1977), and CNS effects (Rowe et al., 1952; Goldbert et al., 1964).

Specific objectives of this study were to: 1) provide accurate direct measurements of the respiratory uptake and elimination of PCE during and following inhalation exposures by simultaneously measuring PCE in the blood and exhaled breath; 2) determine the total dose of PCE absorbed systematically (cumulative uptake) during 2-hour inhalation exposures using inhaled and exhaled breath determinations and the monitored volumes of respiration; 3) validate a physiologically-based pharmacokinetic (PBPK) model for PCE inhalation by

comparing computer simulations of PCE pharmacokinetics with experimentally observed values.

MATERIALS AND METHODS

Animals. Adult, male Sprague-Dawley rats were obtained from Charles River Breeding Laboratories (Raleigh, NC). The animals were maintained on a constant light-dark cycle, with light from 0700 to 1900 hr and darkness from 1900 to 0700 hr. They were housed in stainless-steel cages in a ventilated animal rack. Tap water and Ralston Purina Formulab Chow® were provided *ad libitum*. The rats were used after at least a 14-day acclimation period, at which time their body weight ranged from 325-375 g. Solvent exposures were initiated at approximately the same time each day (1000 to 1200 hr).

Test Material. Perchloroethylene (PER), of 99% purity, was obtained from Aldrich Company Inc. (Milwaukee, WI). The purity of the solvent was verified by gas chromatography.

Animal Preparation. An indwelling carotid arterial cannula was surgically implanted into each animal. The rats were anesthetized for the surgical procedure by im injection of 0.8 ml/kg of a mixture consisting of catamenia HCl (100 mg/ml):acepromazine maleate (10 mg/ml):xylazine HCl (20 mg/ml) in a proportion of 3:2:1 (v/v/v). The cannulated animals were maintained in a harness and pulley system that allowed relative freedom of movement in metabolism cages during a 24-hr recovery period.

Inhalation Exposures. Each cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneve, Switzerland). A face mask designed to fit the rat was held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. A miniaturized one-way breathing valve (Hans Rudolph, Inc.,

St. Louis, MO) was attached to the face mask so that the valve entry port was directly adjacent to the nares of the test animal. This established separate and distinct airways for the inhaled and exhaled breath streams with no significant mixing of the inhaled and exhaled air. The use of such a device for pharmacokinetic studies of inhaled halocarbons in small animals has been described in detail (Dallas et al., 1986). A known concentration of PCE was generated within a 70-liter gas sampling bag (Calibrated Instruments, Ardsley, NY) by injecting the appropriate quantity of the solvent into the bag filled with air. Uniform dispersion of the vapor was insured by a magnetic stirring bar within the bag. The bag was then connected in series by Teflon® tubing with a pneumotachograph, the one-way breathing valve and an empty 70-liter gas collection bag. The latter bag served as a reservoir to collect exhaled gas. The breathing valve was attached to a face mask designed to fit the rat, so that the valve entry port was directly adjacent to the nares of the test animal. Inhalation and exhalation sampling ports were located immediately adjacent to the breathing valve. Each cannulated rat was placed into a restraining tube of the type used in nose-only inhalation exposure chambers (Battelle-Geneve, Switzerland). The face mask was held firmly in place on the animal's head by the use of elastic straps, which were secured to the restraining tube. PCE inhalation exposures of 2 hr duration were initiated only after stable breathing patterns were established. During this exposure period and for up to 4 hr afterward, serial inhaled and exhaled breath samples were taken at approximately the same time as blood samples from the carotid artery cannula. Both air and blood samples were then analyzed for PCE content by headspace gas chromatography.

Respiratory Measurements and Calculations. In order to calculate the total received dose of PCE during inhalation exposures, the respiration of each animal

was continuously monitored. The respiratory monitoring technique was conducted according to the methods previously published in solvent exposure studies by this laboratory (Dallas et al., 1983, 1986, 1989). The airflow created by the animal's inspiration was recording both during and following PCE inhalation exposure in terms of minute volume (volume of respiration per minute, V_E), respiratory rate (f), and tidal volume (V_T). An average value for these parameters for each individual animal was obtained by averaging the measurements taken at 15-min intervals during the 2-hr exposure. The mean ± SD of these average values for the 500 ppm exposure group (n=6) were: V_E = 189 ± 21.5; f = 119.1 ± 22.4; V_T = 1.62 ± 0.34. The mean ± SD of these average values for the 500 ppm exposure group (n=6) were: V_E = 216 ± 43.1; f = 134.5 ± 14.9; V_T = 1.67 ± 0.36.

Calculations of PCE uptake and elimination were conducted utilizing the equations presented in a previous VOC inhalation study in rats (Dallas et al., 1989). Since the V_E and the exhaled breath PCE concentration at each sampling point were measured, subtraction of the quantity of PCE exhaled by the animal from the amount inhaled yielded an estimation of the quantity of PCE taken up during sequential sampling periods (cumulative uptake). The percent uptake during each exposure period was determined by dividing the cumulative uptake by the total inhaled dose for the time period.

The uptake, disposition, and elimination of PCE was described using physiologically-based pharmacokinetic (PBPK) model. The model was used to describe the disposition of PCE in the rat (Fig. 1). It was assumed that a blood flow-limited model was adequate to characterize the tissue distribution of PER. Previous PBPK models for VOCs have utilized blood-flow-limited organ representations (Ramsey and Anderson, 1984; Angelo and Pritchard, 1984; Dallas

et al., 1989, 1991). Compartmental volumes and organ blood flows were obtained from the values used by DELP et al. (1991) for rats, and scaled to 340 g, the mean body weight of rats use din the present study. Metabolism constants were utilized from Chen and Blancato (1987). The alveolar mass transfer coefficient was based on the alveolar permeability-area product for methylene chloride (Angelo and Pritchard, 1987). The lung:air partition coefficient was then derived using the AUC method by Gallo et al. (1985). Differential mass balance equations, incorporating the parameters listed in Table 1, that described the transport of PCE in the rat were numerically integrated with the Advanced Continuous Simulation Language (ACSL) computer program (Mitchell and Gauthier, Concord, MA). The solution to the equations provided predicted PCE concentrations over time. The model-predicted cumulative uptake values were the sum of the simulated amounts of PCE in each tissue compartment in the model.

Analysis of PCE in air and blood. The concentration of TCE in the inhaled and exhaled air samples collected during and following the inhalation exposure were measured with a Tracor MT560 gas chromatograph (Tracor Instruments, Austin, TX). Standards were prepared in each of four 9-liter bottles equipped with Teflon® stoppers with needles from which air samples could be taken by syringe. Analyses for the 500 ppm exposures were conducted using a flame ionization detector (FID), while the analyses for the 50 ppm inhalation exposures were conducted using an electron capture detector (ECD). In either case, air samples were procured with a gas-tight, 1-ml syringe and injected directly onto an 8-ft X 1/8-in stainless steel column packed with 0.1% AT 1000 on GraphPak. Operating temperatures were: 150°C, injection port; 200°C, FID detector; 350°C, ECD detector; 110°C, isothermal column operation. When using the ECD, gas flow rates

of 40 ml/min were employed for nitrogen (carrier gas), with an additional make-up gas flow rate to the detector of 30 ml/min.

Blood levels of PCE were measured by gas chromatographic headspace analysis. Blood samples were withdrawn from the arterial cannula via a stopcock into a 1-ml syringe. Depending on the anticipated blood TCE concentration, from 25 to 200 μl for the blood was taken from the stopcock with an Eppendorf pipette and transferred to chilled headspace vials (Perkin-Elmer, Norwalk, CT). These vials were capped immediately with PTFE-lined butyl rubber septa nd washers and tightly crimped. Each sample vial was then placed into the HS-6 auto-sampler unit of a SIGMA 300 gas chromatograph (Perkin-Elmer, Norwalk, CT), where it was heated to a present temperature by a high precision thermostat device. A predetermined volume of the vapor was then injected automatically into the column for analysis. The column used was an 8-ft X 1/8-in stainless-steel column packed with 10% FFAP chromasorb W-AW (80-100 mesh). Operating temperatures were: 200°C, injection port; 400°C, ECD detector; and 110°C column oven. The carrier gas was 5% argon-methane, at a flow rate of 40 ml/min, with a make-up gas flow rate of 20 ml/min to the detector.

RESULTS

With target concentration of 50 and 500 ppm for the PCE inhalation exposure, the actual concentrations inhaled by the animals were determined by analysis of air samples taken from the airway immediately adjacent to the breathing valve. Inhaled PCE concentrations for the six rats in each group were 52.8 ± 2.2 ppm ($\bar{x}$ ± SD) for the 500 ppm exposure sand 53.1 ± 5.1 ppm ($\bar{x}$ ± SD) for the 50 ppm exposures.

Significant respiratory elimination of unchanged PCE was evident during the inhalation exposure period, with near steady-state PCE levels achieved in the exhaled breath within 20-30 min. These near-steady state concentrations were approximately 2.1-2.4 μg/ml in the exhaled breath of the 500 ppm exposed rats (Fig. 2). In the 50 ppm inhalation exposure group, these exhaled breath levels at near-steady state were in the range of 0.20-0.22 μg/ml (Fig. E-3).

PCE was rapidly absorbed from the lungs and readily available for distribution to tissues of the body, in that arterial blood concentrations of PCE were quite high at the first sampling time (i.e., 2 min). Unlike the exhaled breath data, the concentration of PCE in the blood progressively increased over the course of the 2-hr exposure in both exposure groups. The rate of increase in PCE concentration in the blood was greater in the 500 ppm (Fig. E-4) than in the 50 ppm group (Fig. E-5). Arterial PCE concentrations were not proportional to the inhaled concentration. After the initial rapid uptake phase over the first 30 minutes of exposure, blood levels in the 500 ppm rats were 12 to 17 times higher than 50 ppm rats. Upon cessation of PCE inhalation, the chemical was rapidly eliminated. As can be seen in Figs. 3 and 4, PCE concentrations in the exhaled breath initially diminished more rapidly than did blood concentrations. Disappearance of PCE from the blood paralleled that in the expired air during the latter pair of the postexposure period. The PCE levels were not monitored long enough postexposure to accurately define the terminal elimination half-lives.

Measurement of the total cumulative uptake of PCE by the rats was made by accounting for the quantity of unchanged PCE tat was exhaled during the inhalation exposure period. The total cumulative uptake of PCE from the 2-hr exposure to 500 ppm (Fig. 6) was 28.1 ± 4.3 mg (x ± SD), or 79.9 mg/kg bw. The

2-hr exposure to 50 ppm PCE (Fig. 7) resulted in a cumulative uptake of 3.9 ± 0.9 mg (x ± SD), or 11.2 mg/kg bw. Predicted values for uptake, derived by summating the predicted levels of PCE in the model compartments, were significantly less than these measured uptake values (i.e., after 2 hr exposure to 500 ppm PCE predicted uptake was 80% of measured uptake). Percent systemic uptake of PCE is shown in Figure 7. Although percentage uptake was quite high during the initial minutes of inhalation of 50 and 500 ppm TRI, a decrease of 20-25% occurred during the first hour. Thereafter, there was relatively slow decline in uptake for the remainder of the 2-hr exposure.

The PBPK model predictions of blood and exhaled breath concentrations of PCE are shown in Figures 2 and 3. Both observed and predicted exhaled breath concentrations of PCE rapidly achieved a steady state following the initiation of exposure. The concentrations of PCE in the exhaled air during 50 ppm and 500 ppm inhalation exposure were well predicted relative to the observed concentrations, with a slight underprediction by the model post-exposure. The rapid uptake of PCE in the blood during the first four of 50 ppm inhalation was well predicted with a slight overprediction thereafter. The steep increase in blood concentration of PCE during 500 ppm inhalation exposure was accurately predicted by the model simulations. PCE blood concentrations post exposure were also well simulated, with slight underpredictions starting at one hour post exposure.

The PBPK model was also utilized to generate predictions of cumulative and percent uptake of PCE during inhalation exposure, which were compared to values determined from direct measurement in figures 3 and 4. Cumulative uptake of PCE was well predicted over the course of the 500 ppm inhalation exposure. During 50 ppm exposure, through, the magnitude of the degree of underprediction by the

model increased steadily over the course of the 2 hr exposure. Percent uptake was slightly underpredicted during both 50 and 500 ppm exposure to PCE. In the second hour of 500 ppm exposure model predictions of percent uptake were within 2% of the observed values. For the 50 ppm exposure, the predicted percent uptake was consistently about 10% below the observed percent uptake.

DISCUSSION

As is characteristic for many volatile halocarbons, the major route of elimination for PCE in laboratory animals and man is by exhalation of the parent compound. As with trichloroethylene (TCE) and trichloroethane (TRI), the pharmacokinetic data base for PCE is unique in that there have been several studies published with direct measurements of the elimination of the halocarbon in humans. This allows a direct interspecies comparison of the measurement of the halocarbon eliminated in the exhaled breath between laboratory animals and man. Fernandez et al. (1976) measured PCE in the exhaled breath of human volunteers following exposures ranging from 1 to 8 hours to controlled concentrations of PCE in dynamic exposure chambers. Following exposure to 100 ppm for 2 hours, the PCE concentration in the exhaled breath at 1 and 2 hrs post-exposure were 0.06 and 0.047 μg/ml. Assuming a linear scale up of the 50 ppm data from the current investigation, the expired air PCE concentration in the rats at these two time points would be 0.07 and 0.04 μg/ml, respectively. In comparisons where differences in exposure concentration are accounted for, the postexposure PCE exhaled breath levels in several other human studies (Stewart et al., 1961; Stewart et al., 1970) were also similar in magnitude to the measurements of PCE in the expired air of rats in this current study. This pattern of a similarity in the concentrations of halocarbon eliminated in the exhaled breath of rats and humans was also noted for TRI (Dallas et al., 1989)

and TCE (Dallas et al., 1991). Since the blood:air partition coefficient for PCE, TRI, and TCE is markedly higher in rats than in humans (Gargas et al., 1989), it would be anticipated that this physicochemical difference would result in a greater magnitude of halocarbon respiratory elimination by humans.

As PBPK models for PCE have been developed in recent years, there has been an emphasis in the application of these modeling approaches to human risk assessment. The amount of PCE metabolized by animals that showed tumor responses in cancer bioassays was predicted using a PBPK model (Chen and Blancato, 1987). Scaling up the predictions of metabolite formation to humans and employing the dose response relationship in the animal studies was then used to calculate human carcinogenic risk. In a unique application of an inhaled dose calculation, the respiratory exposure of humans to PCE in indoor air (resulting from PCE volatilized indoors from contaminated ground water) was determined and employed as the dose input for a PBPK model for PCE in humans (Bogen and McKone, 1988). These authors did not first make an animal model in their development of a human risk assessment using calculations of metabolite formation. Using a PBPK model for PCE as an example, Farrar et al. (1989) characterized the degree of uncertainty in the model output and human risk estimates due to uncertainty in model parameters. The rate of PCE metabolite formation in mice, rats, and humans was predicted using a PBPK model and compared to observed data available in the literature (Bois et al., 1990). Hattis et al. (1990) have compared the predictions of metabolite formation from PCE from the different published models. These studies did not make comparisons of the PBPK model predictions of parent compound uptake or disposition with experimentally derived data, as the emphasis was on metabolite formation and risk assessment.

In the current study, the emphasis was on comparisons of the predictions of the PBPK model to direct observations of a number of pharmacokinetic parameters of the parent compounds for verification of the validity and utility of the model. Total cumulative uptake of the PCE over time was well simulated over the course of 500 ppm inhalation, and underpredicted during 50 ppm exposure. Predictions of unchanged PCE in the expired air of rats following exposure to inhaled PCE were well simulated by a PBPK model by Ward et al. (1988) in comparison to previously published data (Pegg et al., 1979). The authors increased the fat-air partition coefficient from the measured value of 1638 to 2300 in order to account for the concentrations of PCE in the post-exposure expired air. In the present investigation, the partition coefficient for fat:blood derived from the tissue AUC values was 109, which corresponds to a fat:air coefficient of 2881. While published values of PCE exhaled air and blood concentrations were available during inhalation exposure in humans (Fernandez et al., 1976) for use in comparison to model predictions by Ward et al. (1988), no published values were available for these parameters during PCE inhalation in rats. In the current investigation, exhaled air concentrations during exposure to PCE in rats were well predicted by the PBPK model employed. PCE determination in the blood of rats during inhalation exposure also were not previously available for verification of PBPK model predictions. Values for the uptake and elimination of PCE in rat arterial blood in the current study were similar in magnitude and pattern to the prediction by the PBPK model, with some slight overprediction in 50 ppm exposure group.

The ability of the PBPK model to predict uptake, disposition, and elimination of PCE both during and following inhalation exposures increases the confidence in the utility of the model in health risk assessments. In risk

assessments of CNS depression in workers due to inhalation exposures in occupational environments, the concentration of PCE as parent compound would be a relevant dose surrogate of interest. Ward et al. (1988) have demonstrated that the available direct measurements from human studies were well simulated by a PBPK model similar to the one employed in the current investigation. Studies of the disposition of PCE tissue concentration-time data during and following inhalation exposures would further expand the degree of certainty in model predictions for risk assessment purposes.

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TABLE 1

Parameters for the Physiological Pharmacokinetic Model for PCE in the Rat

Parameter	Value
Alveolar Ventilation Rate (ml/min), V_a	115.3 (50 ppm exposure) 101.4 (500 ppm exposure)
Inhaled Gas Concentration (mg/ml)	0.351 (50 ppm exposure) 3.55 (500 ppm exposure)
Alveolar Mass Transfer Coefficient	500 ml/min
Blood Flows (ml/min)	
Cardiac output, Q_b	117.0
Fat, Q_f	8.1
Liver, Q_{Li}	18.1
Muscle, Q_m	32.7
Brain Q_{br}	2.6
Heart Q_h	6.0
Kidney Q_k	16.4
Rest of body Q_r	33.1
Tissue Volumes (ml)	
Alveolar	1.9
Blood	25.5
Fat	24.8
Liver	11.7
Lung	1.32
Muscle	121.2
Brain	2.0
Heart	1.12
Kidney	2.65
Rest of body	153.6
Partition Coefficients	
Lungs:Air	46.87
Fat:Blood	152.54
Liver:Blood	4.96
Muscle:Blood	2.98
Lung:Blood	2.48
Brain:Blood	4.24
Heart:Blood	2.68
Kidney:Blood	4.45
Rest of body:Blood	2.98
Metabolism Constants	
V_{max} (μg/min)	5.86
K_m (μg/ml)	2.938

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INDEX TERMS

Perchloroethylene

Physiologically-based Pharmacokinetic Model

Respiratory Elimination

Pharmacokinetics

Inhalation Exposure

FOOTNOTES

[1] Research sponsored by the Air Force Office of Scientific Research, Air Force Systems Command, USAF, under grant numbers AFOSR 870248 and 910356. The US Government is authorized to reproduce and distribute reprints for Governmental purposes notwithstanding any copyright notation thereon. This manuscript is submitted for publication with the understanding that the US Government is authorized to reproduce and distribute reprints for Governmental purposes.

[2] Presented at the 28th Annual Meeting of the Society of Toxicology, Washington, DC, February 1989.

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FIGURE LEGENDS

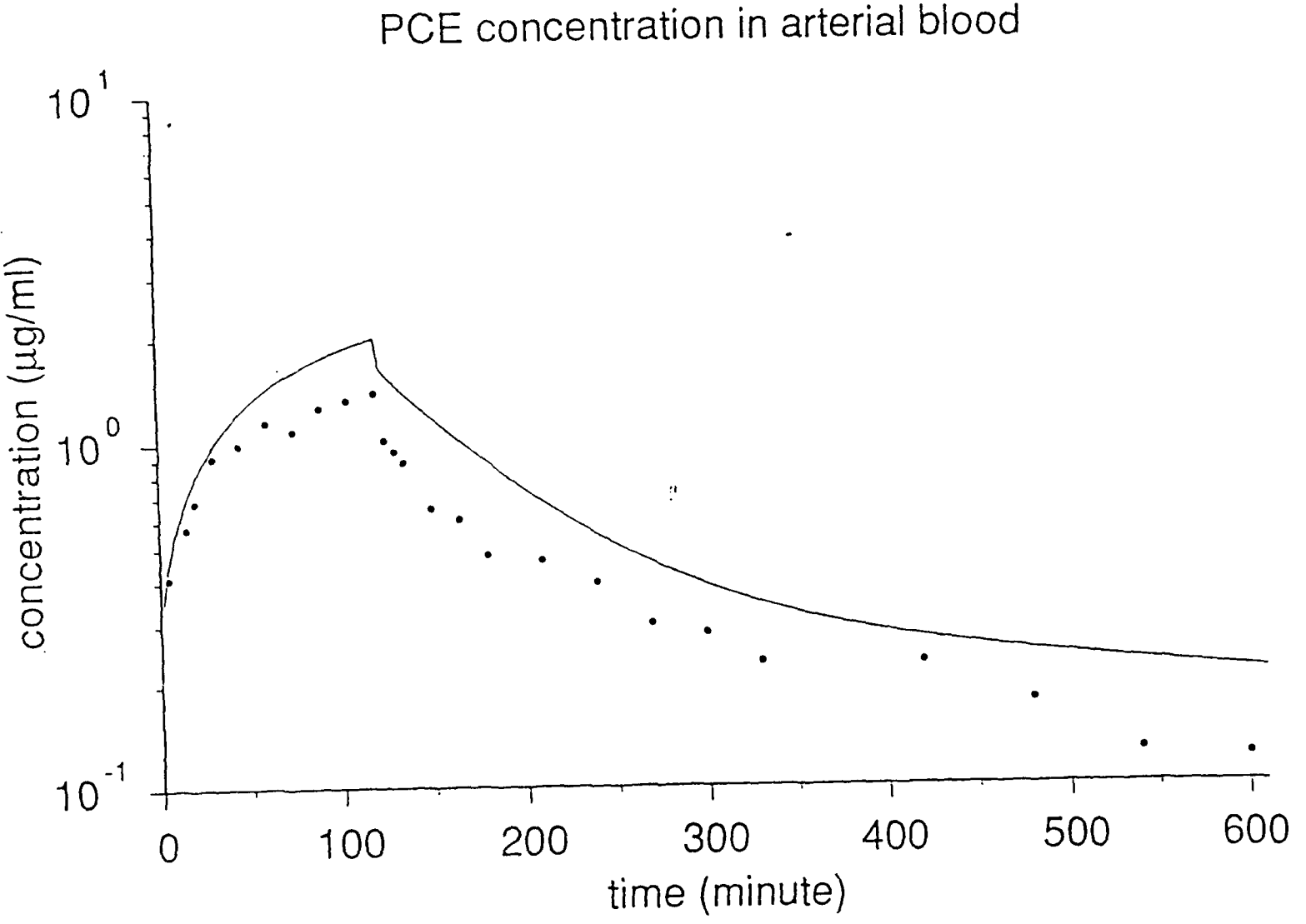
1. Diagram of the physiological pharmacokinetic model used to simulate the uptake and elimination of inhaled PCE. The symbols and parameters used to describe the model are included in Table 1.

2. Observed (•) and model-predicted (-) PCE concentrations in the arterial blood and exhaled breath of rats during and following a 2-hr, 50 ppm inhalation exposure. Each point represents the mean value for 6 rats.

3. Observed (•) and model-predicted (-) PCE concentrations in the arterial blood following a 2-hr, 500 ppm inhalation exposure. Each point represents the mean value for 6 rats.

4. Percent systemic uptake of PCE over time during inhalation exposures to 50 (a) or 500 (b) ppm PCE for 2 hr. Each point represents the mean ± SE for 6 rats. Percent uptake of the inhaled dose was determined after 1, 3, 5, 10, 15 and 20 min and at 10-min intervals thereafter. Model predictions of percent uptake are delineated by the solid line.

5. Cumulative uptake of PCE during inhalation exposures to 50 (a) or 500 (b) ppm PCE for 2 hr. The quantity of inhaled PCE retained during successive 10-min intervals was calculated on the basis of the measured minute volume and difference between inhaled and exhaled PCE concentrations. Each point represents the mean ± SE for 6 rats. Model predictions of cumulative uptake are delineated by the solid line.



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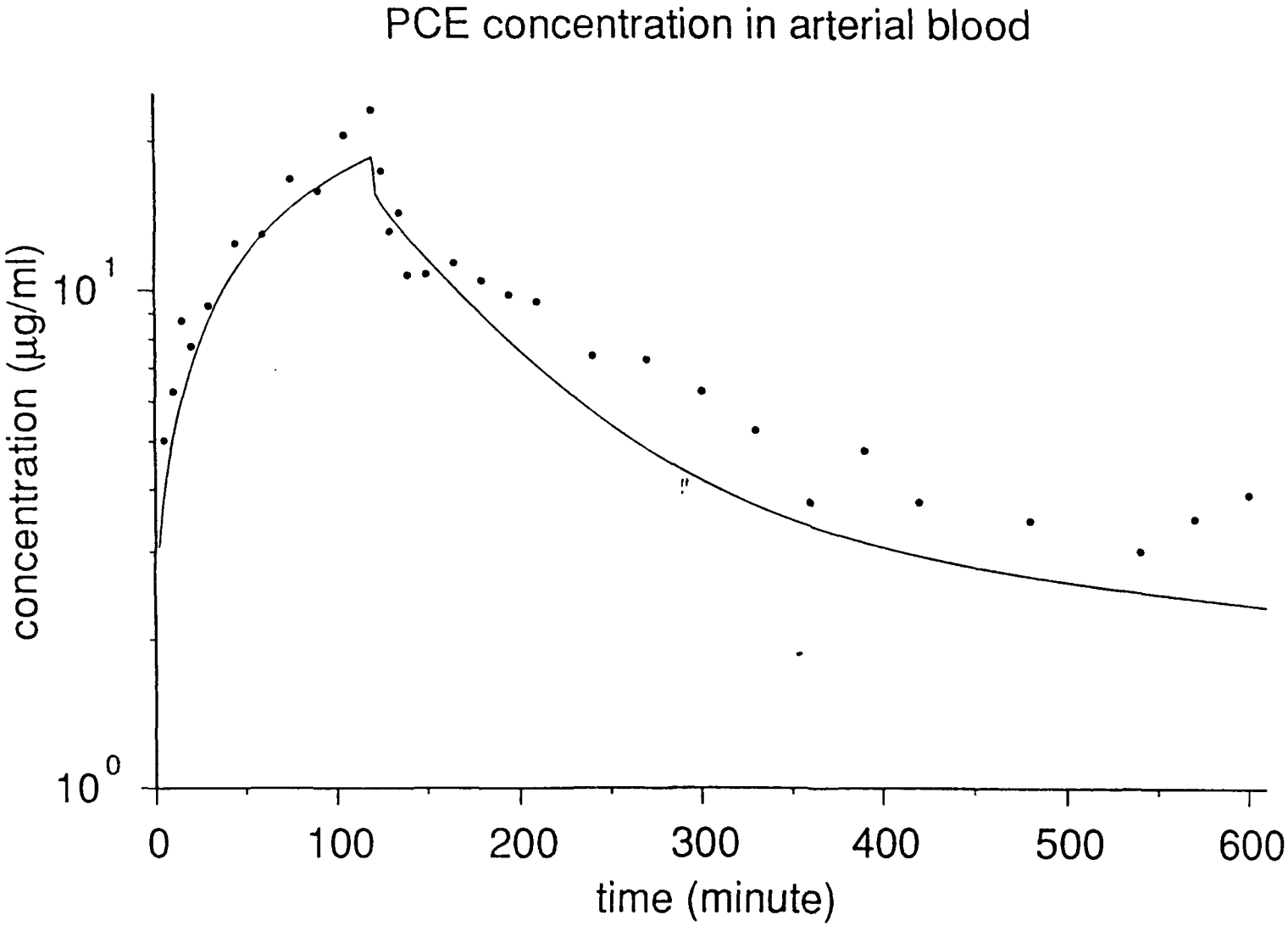

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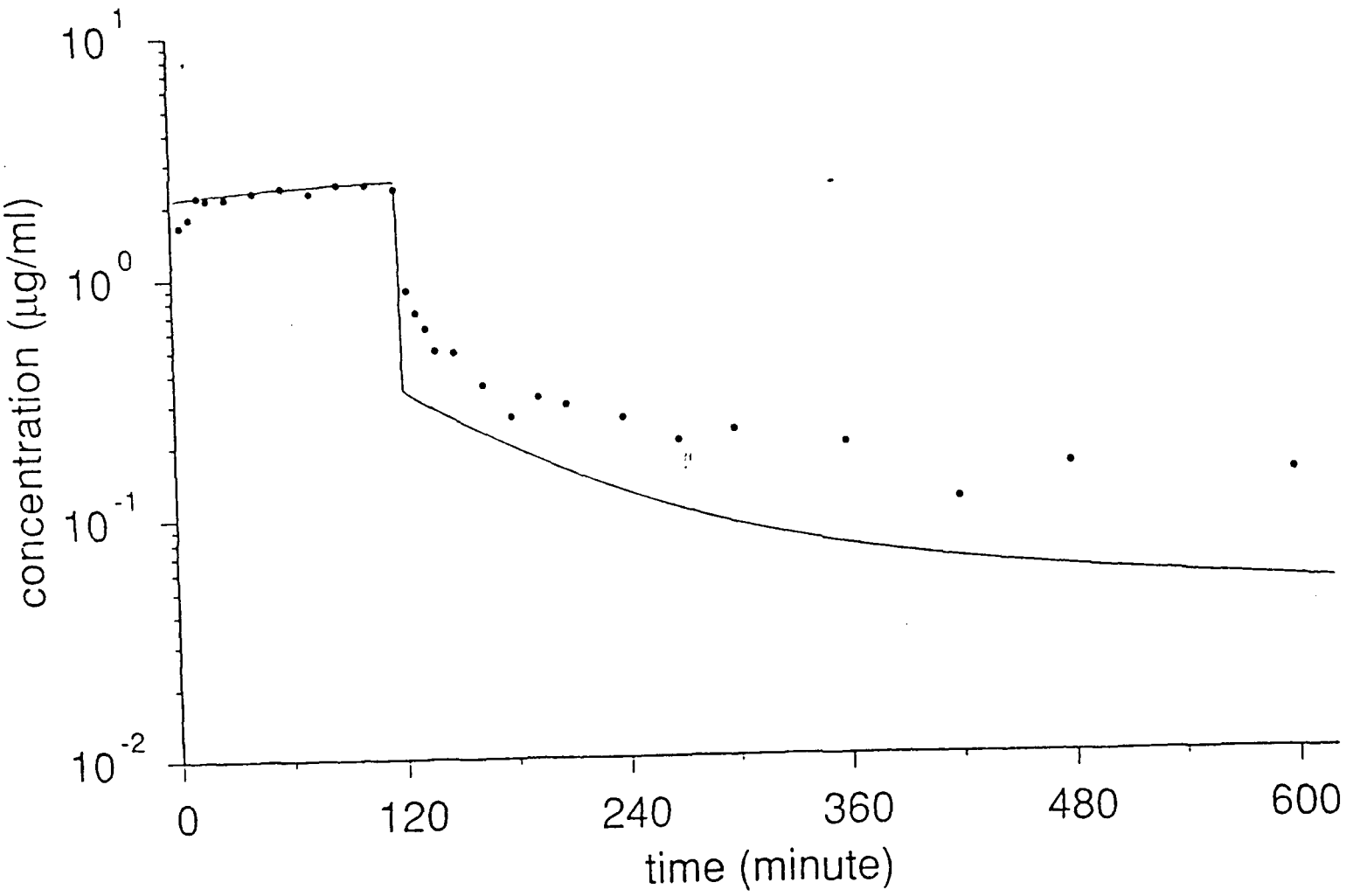

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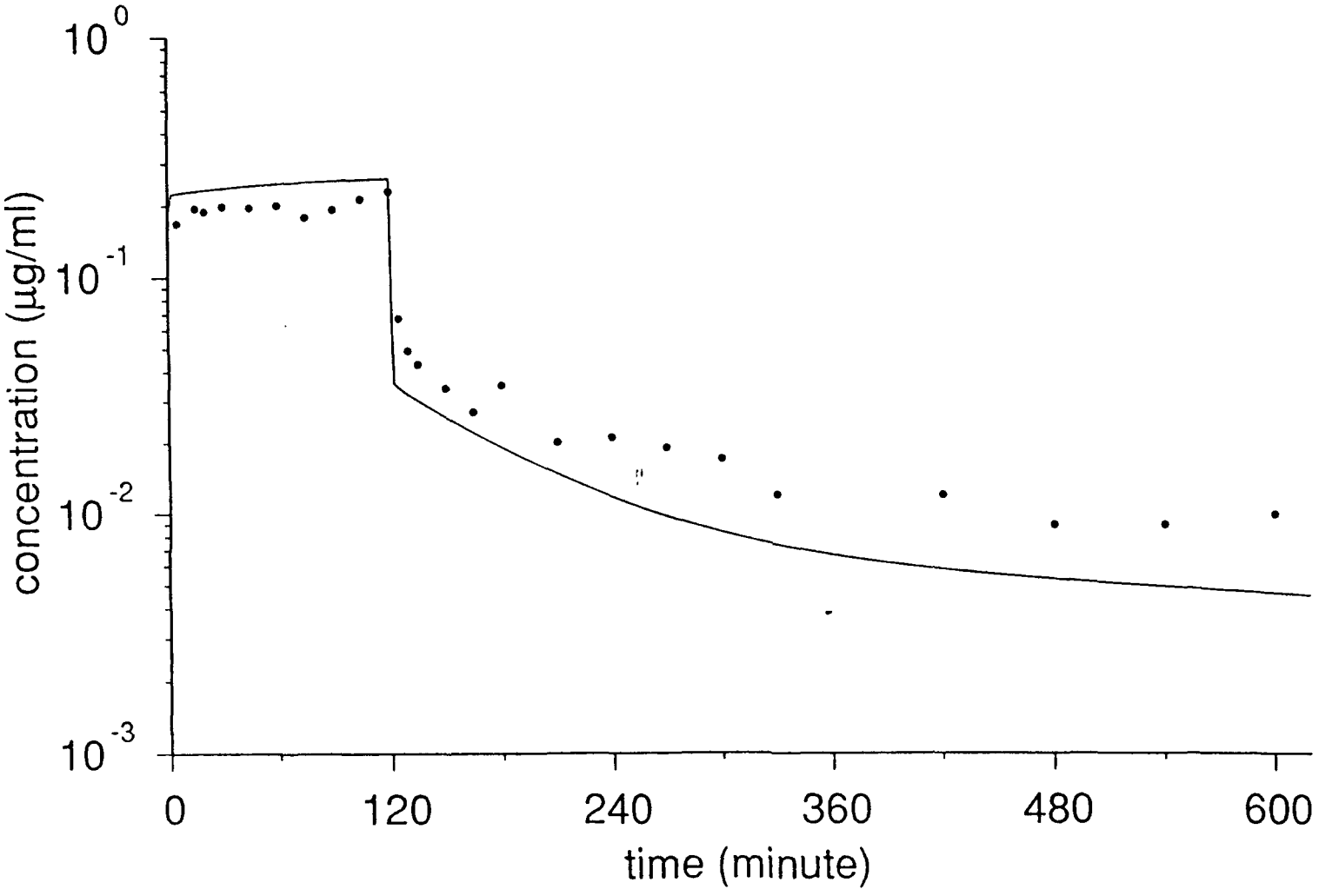

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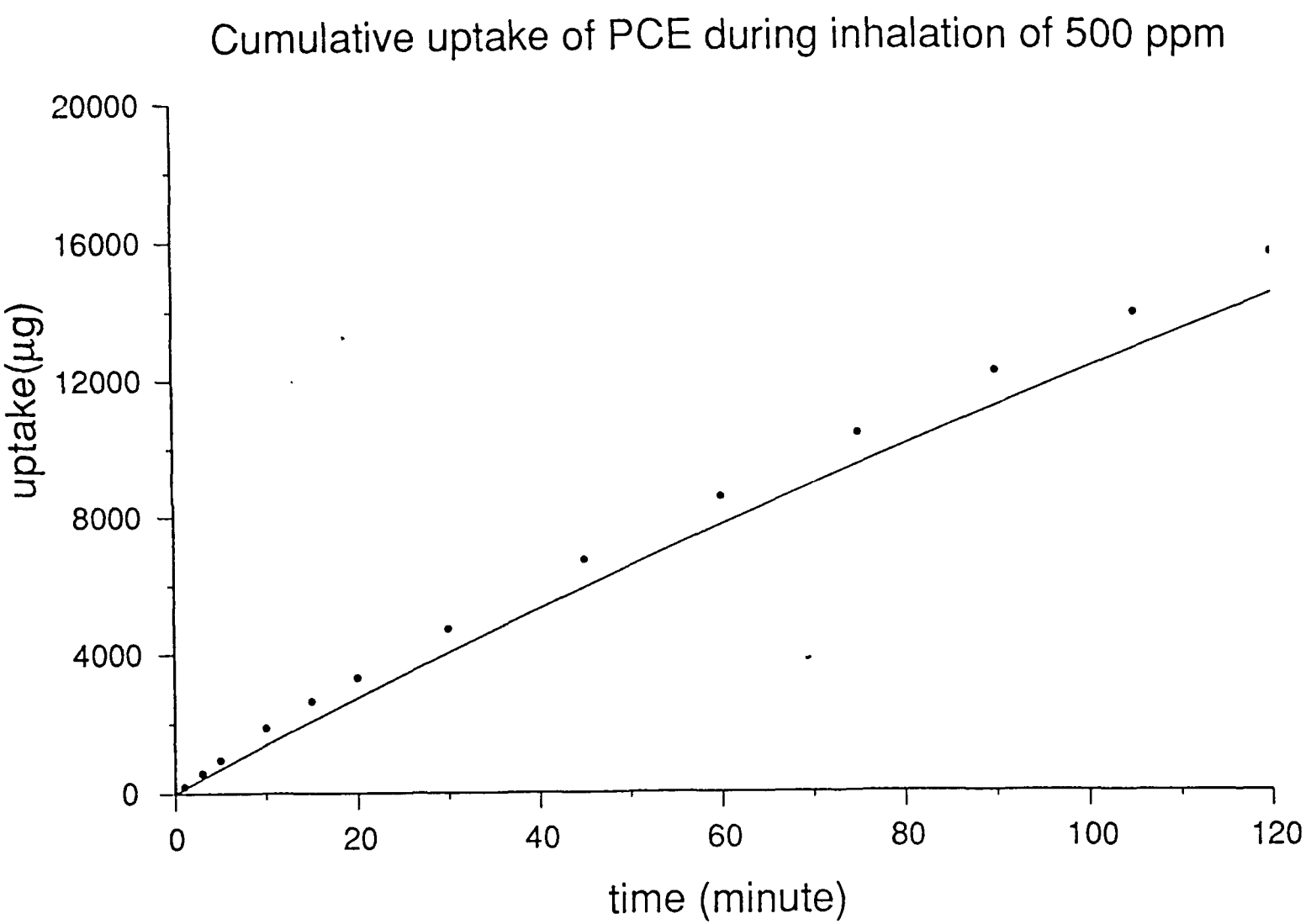
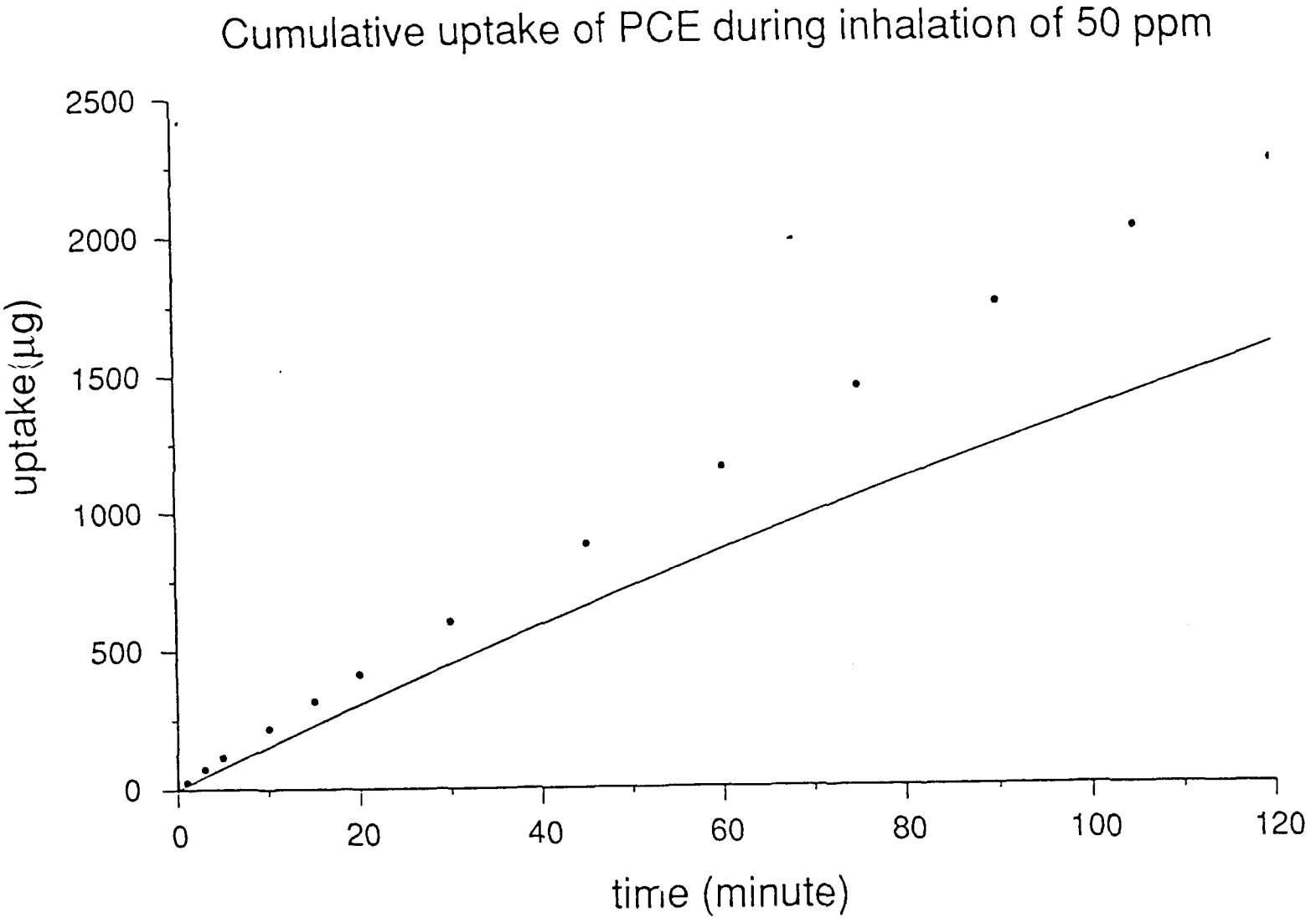
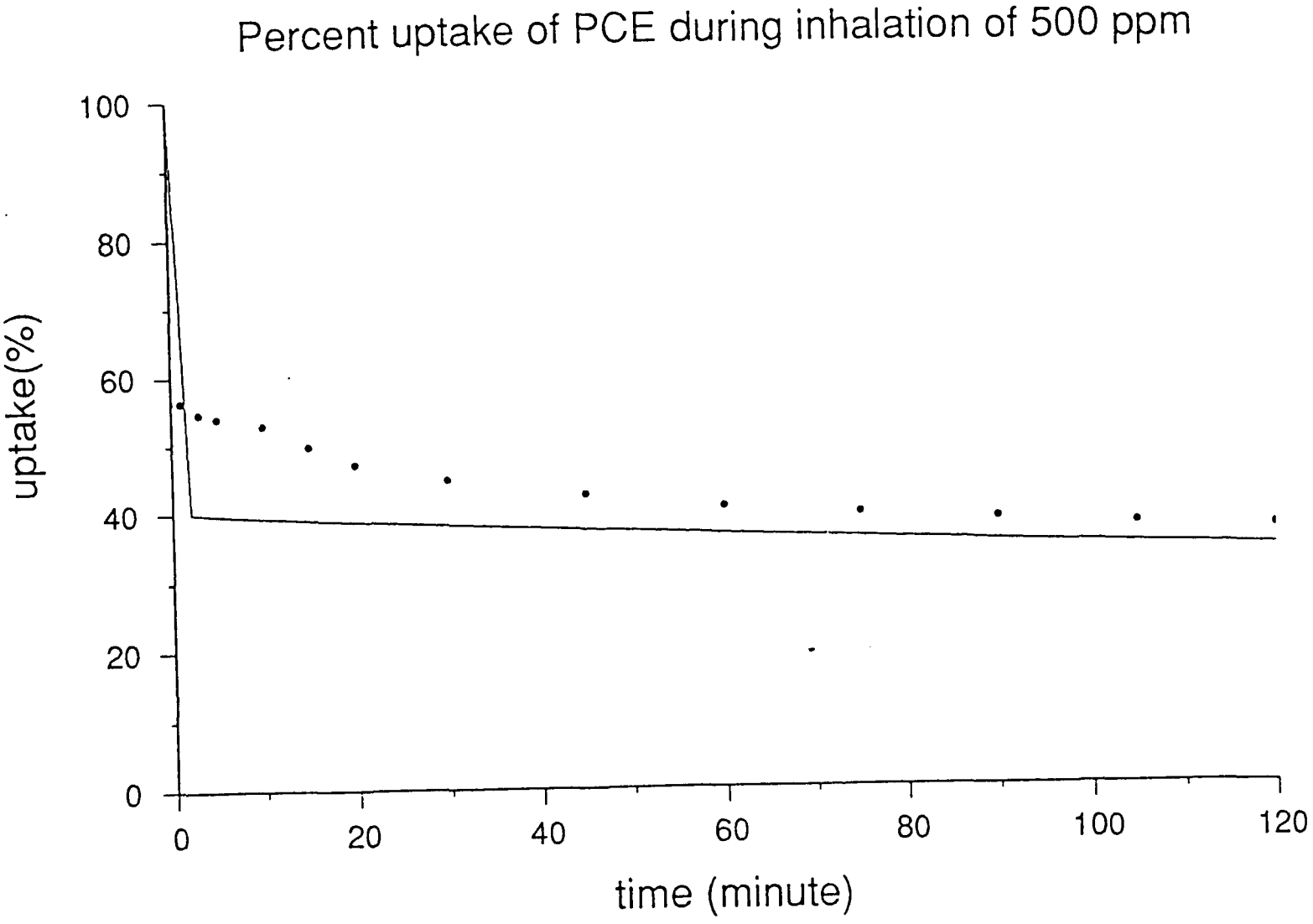
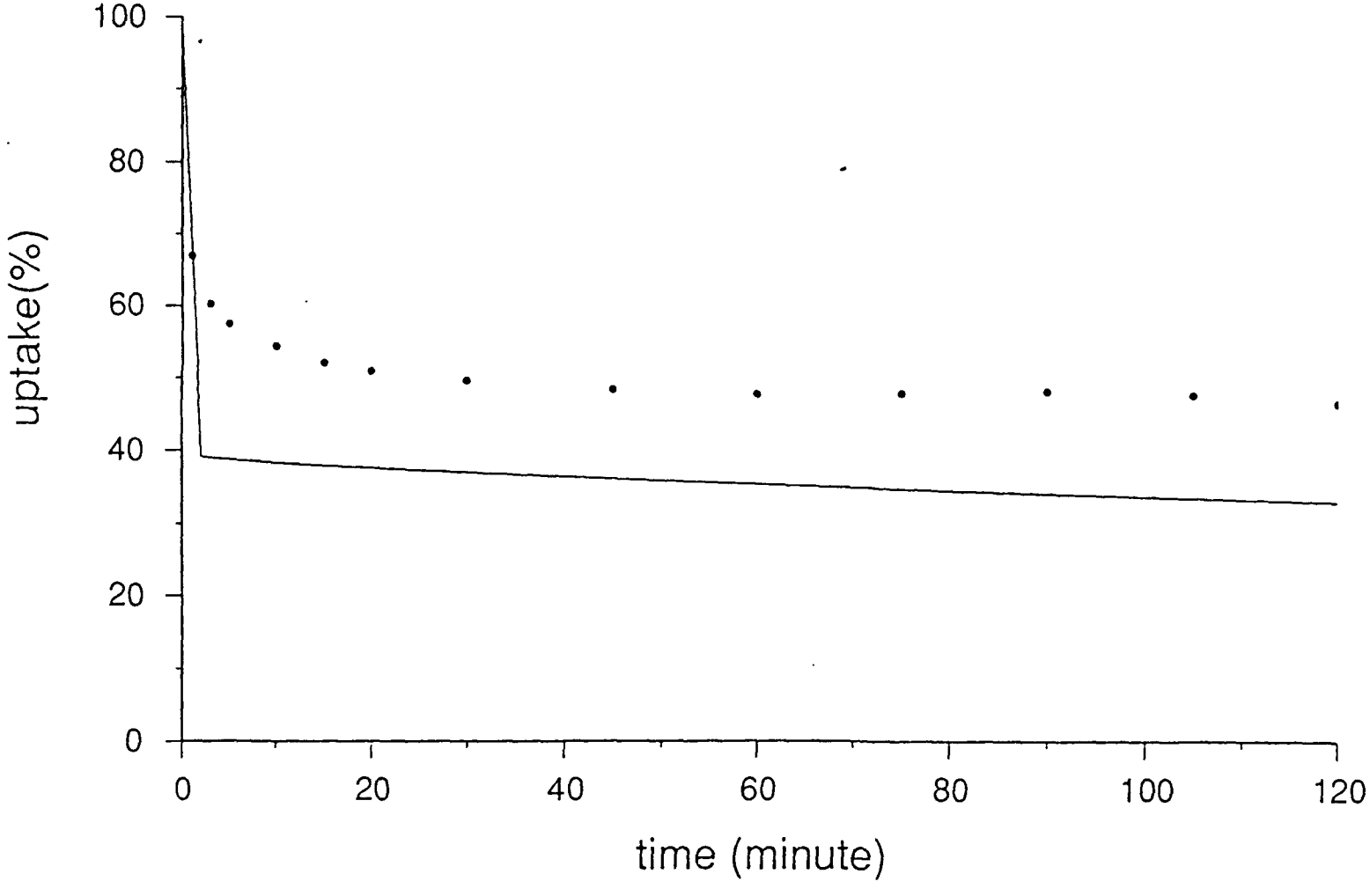

Cumulative uptake of PCE during inhalation of 500 ppm
uptake(µg)
20000
16000
12000
8000
4000
0
0
20
40
60
80
100
120
time (minute)


Cumulative uptake of PCE during inhalation of 50 ppm
uptake(μg)
2500
2000
1500
1000
500
0
0
20
40
60
80
100
120
time (minute)


Percent uptake of PCE during inhalation of 500 ppm
uptake(%)
100
80
60
40
20
0
0
20
40
60
80
100
120
time (minute)


Percent uptake of PCE during inhalation of 50 ppm
uptake(%)
100
80
60
40
20
0
0
20
40
60
80
100
120
time (minute)